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OUTDOOR BUBBLING CULTURE OF SOME UNICELLULAR ALGAE

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INTRODUCTION

Among the various methods thus far attempted to grow unicellular algae outdoors, the "open bubbling method" devised by Gummert *et al.* (1) seems to be the simplest in both its principle and way of operation. The principle of this method consists in growing algae in a shallow pond under constant aeration with CO_2-enriched air which is supplied from the bottom of the pond. Using a strain of *Scenedesmus*, the German workers obtained by this culture method average daily yields ranging from 2.2 (in November) to 6.6 (in August) g/m^2-day, the culture being discontinued during the winter months, December, January and February. Judging from these figures, the efficiency of this culture method appears to be considerably lower than that of "closed circulation method" attempted by American workers (2) who obtained a maximum yield of 11 g/m^2-day (in July) using *Chlorella pyrenoidosa* as material.

The present report deals with a new device of open bubbling culture similar in principle to that of Gummert *et al.* Experiments conducted during the period from August 1954 to June 1955 showed that the culture could be run continuously throughout the year, provided that the algal strains of different temperature-tolerance were used according to the seasons, and that thereby the yields which were appreciably higher than those reported by earlier workers could be obtained.

PRELIMINARY EXPERIMENT WITH A FLAT-BOTTOMED POND

In the first attempt we made of the open pond culture, we used a flat-bottomed trench which is illustrated in Figs. 1 and 2. It was 18 m in length, 40 cm in depth, and had a width of 150 cm at the top and 100 cm at the bottom. The earth was lined with sandy soil hardened with bittern-salt and covered with vinyl sheeting of 0.2 mm in thickness. Three rows of vinyl pipes (1.7 cm in diameter) having small holes (1.0 mm in diameter) at a distance of 20 cm were laid in parallel at the bottom of the trench, and through these holes CO_2-enriched air was bubbled into the culture day and night at a rate of 70 ml per minute per liter of culture.* The culture

* This was the upper limit of the rate of gas supply by our equipment at the time of running these experiments.

solution used was 3500 (3000–4000) liters in volume, 15–20 cm in depth, and 22 m^2 in illuminated surface. The trench was first covered with a framed roof of vinyl sheeting to protect the culture from dust and to prevent, as much as possible, the escape of carbon dioxide from the solution; however, in view of many troubles caused by strong wind we finally abandoned this covering and the culture was left open in most of the experiments.

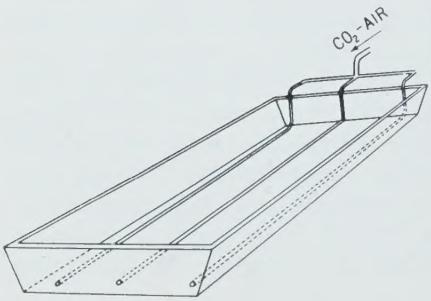

Fig. 1. Schematic diagram of the flat-bottomed culture pond.

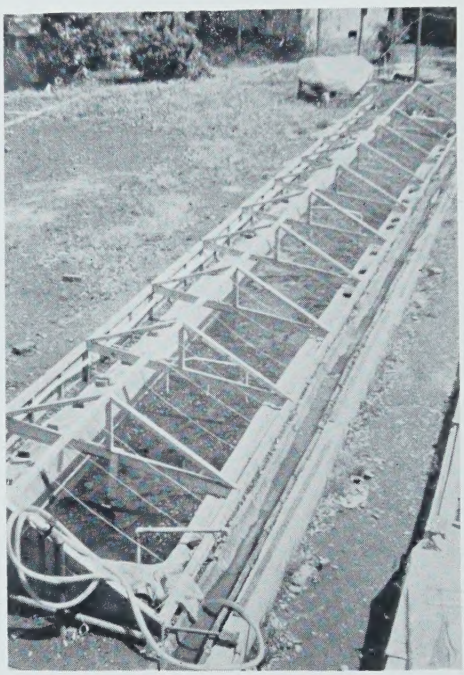

Fig. 2. Flat-bottomed culture pond. The frame on the top was for covering the pond with vinyl sheeting, which was, however, not in use in most of the experiments.

A number of experiments run with different strains of *Chlorella* and *Scenedesmus* showed that the construction of the trench was inappropriate, the most serious defect being the insufficiency of agitation of culture, which entailed precipitation of a large quantity of algal cells between the rows of aeration pipes. This precipitation not only decreased the rate of algal growth, and made the procedure of harvesting difficult, but also became a serious cause of contamination by micro-animals such as *Protozoa* and *Rotifera*. In fact, many of our experiments had to be discontinued within a week or so (or even within 3 days in the worst case) owing to the deterioration of algal cells caused by contamination. To save space, we shall not present here at length the records of those experiments which had yielded erratic results. A relatively successful experiment using a strain of *Chlorella*,

which could be continued for 17 days in June showed that the average yield was only 6.0 g/m^2-day*, a rate which was less than half of the expected value**, 15 g/m^2-day. Another experiment using *Scenedesmus* sp., which was continued for 20 days in May gave an average yield of 5.0 g/m^2-day, whereas the expected value was as much as 9.0 g/m^2-day.

DEVICE OF NEW PONDS

Considering the difficulties encountered with the flat-bottomed pond, we designed a pond with zigzag-formed bottom, which is schematically illustrated in Fig. 3. The pond was provided with a number of ridges (triangular prism in form) at the bottom, and from the plastic pipes placed in the hollows between the ridges CO_2-enriched air was constantly bubbled into the culture.

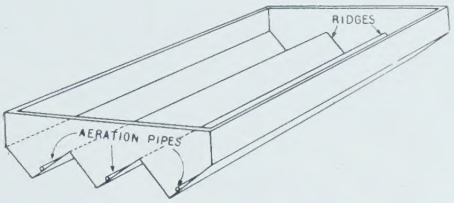

Fig. 3. Schematic diagram of the zigzag-bottomed pond.

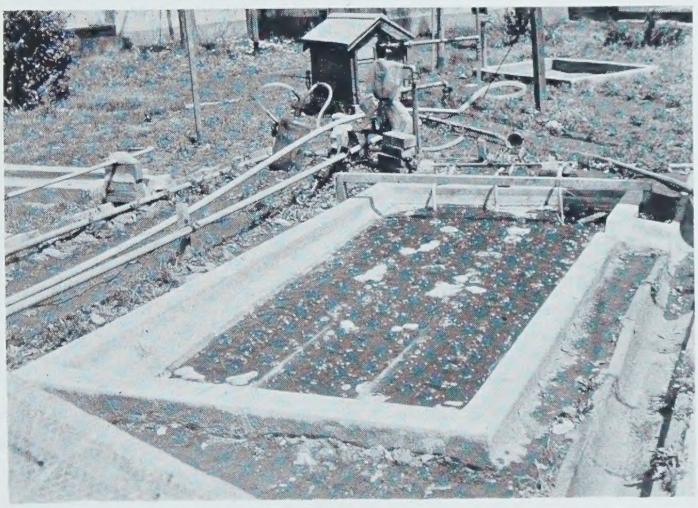

Fig. 4. An example of zigzag-bottomed open ponds.

* The sampling of culture for determining the growth rate was done every day after making the culture homogeneous by sweeping and raising the precipitated cells.

** The "expected values" are those determined with control cultures (using flat culture bottles described in a previous paper (3)), which were placed outdoors under natural conditions. Details on this control culture, which gave us useful informations regarding the possible yield of algae in different seasons, will be reported elsewhere (4).

The existence of ridges between the aeration pipes had the double purpose of increasing the ratio of illuminated surface to the volume of culture, and making the agitation of culture as effective as possible. The pond was filled with culture solution in such a quantity that the tops of the ridges were just covered by the surface of the solution (Fig. 4).

By preliminary tests made with a number of small trenches (Fig. 5) and culture chambers (Fig. 6) having V-shaped bottoms of different angles, we learnt that in order to prevent the precipitation effectively:

Fig. 5. An example of small trenches having V-shaped bottoms of different angles.

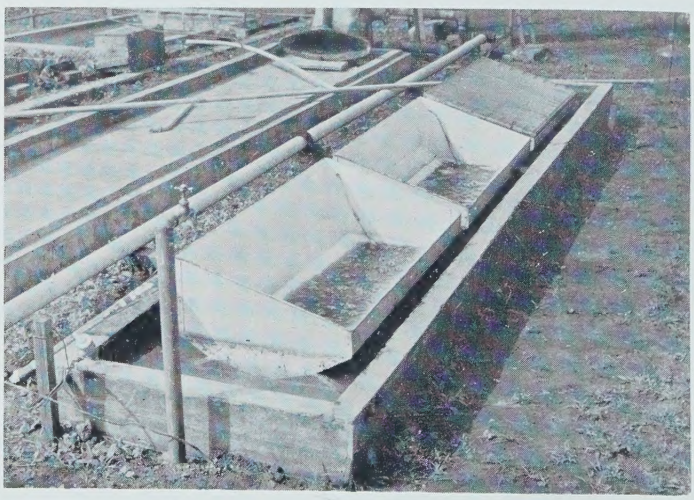

Fig. 6. An example of culture chambers having V-shaped bottoms. On rainy days they were covered with framed vinyl sheeting.

(1) the angle of ridges must be in the range between 90° and 105°,
(2) the depth of the solution at the center of the hollows should be made about 20 cm or less,
(3) the distance between the aeration pipes should be less than about 60 cm, and the interval of holes on the pipes be less than about 15 cm,
(4) the rate of aeration should be greater than 200 ml per minute per liter of culture,
(5) as regards the lining material of the pond, vinyl sheeting appeared to be the best, although uncovered concrete or asphalt as well as the concrete painted with vinyl paint also proved to be usable.

Based on these experiences we constructed 4 culture ponds of the above-mentioned type, the features of which are summarized in Table 1.

Table 1. The features of the ponds used in the main experiments

Pond No.	Number (& angle) of ridges	Material	Volume of culture solution (liters)	Area of illuminated surface (m^2)	Average (& maximum) depth of culture (cm)	Distance between aeration pipes (cm)
I	2 (90°)	Concrete lined with vinyl sheeting	400	3.5	11.5 (22)	38
II	3 (95°)	Concrete vinyl-painted	300	2.8	11 (16.5)	40
III	3 (95°)	Concrete vinyl-painted	500	5.5	9 (16)	45
IV	1 (105°)	Dug earth covered with vinyl sheeting	200	2.5	8 (15)	52

All ponds were provided with vinyl pipes (1.7 cm in diameter) having holes (directed downward, 1 mm in diameter) at intervals of 15 cm. Through these holes air containing 2.5–4 per cent CO_2 was constantly (day and night) bubbled into the solution at a rate of about 250 ml per minute per liter of culture.

RESULTS AND DISCUSSION

The experiments lasted from August 1954 to June 1955. During the summer months we used five temperature-tolerant strains*, *Chlorella* Ctm37, Ctn1, Ctn6 and Ctn7, and *Scenedesmus* Stm32, and in other seasons *Chlorella ellipsoidea*, Cn2, Cn3, Cn4, and *Scenedesmus* Sm36 which had ordinary temperature-tolerance. The culture solutions used had the following composition, per liter:

* These strains had been isolated from pond waters in or near Tokyo. They all tolerated temperatures as high as 42°C, but ceased to grow in colder months, December to February.

Urea 3.0 g (or KNO_3 5.0 g), $MgSO_4 \cdot 7H_2O$ 2.5 g, KH_2PO_4 1.25 g, $CaCl_2 \cdot 2H_2O$ 22 mg, $ZnCl_2$ 8.3 mg, $CoCl_2 \cdot 6H_2O$ 0.8 mg, $MnSO_4 \cdot 5H_2O$ 3.3 mg, $CuSO_4 \cdot 5H_2O$ 3.1 mg, $Na_2B_4O_7 \cdot 10H_2O$ 35 mg, $3(NH_4)_2O \cdot 7MoO_3 \cdot 4H_2O$ 1.5 mg, $FeSO_4 \cdot 7H_2O$ 10 mg, Ethylenediamine tetraacetic acid (disodium salt) 7.4 mg.

Prior to the main culture, the algae were grown either outdoors in the small trenches or culture chambers shown in Figs. 5 and 6, or indoors in large glass cylinders provided with fluorescent day-light lamps. The preculture lasted 7–10 days and the thick cultures thus obtained were used as the seeding material for the main outdoor cultures. The initial population density in the main culture was 0.3–0.5 ml (packed cell volume) per liter.

The four ponds used seemed to have no essential difference in their usability and efficiency. The growth of algae was followed by measuring the packed cell volume per liter every day or at intervals of 2–3 days. Taking into account the total volume of culture, the area of illuminated surface and the ratio of dry weight to packed volume of cells, the growth rate was compared in terms of gram (dry weight) per square meter per day. Sampling of culture was made every time after thoroughly mixing the whole culture, and, if necessary, raising the cells which occasionally precipitated on the surface of ridges.

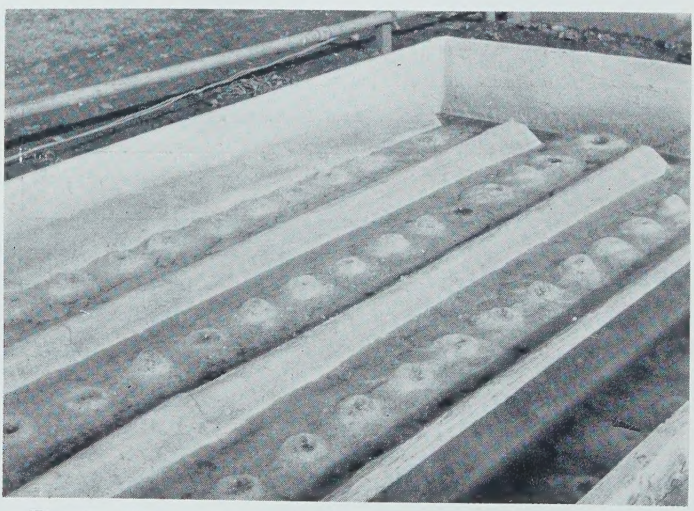

Fig. 7. The pond covered with ice in early winter mornings. It melted after sunrise and never lasted until noon.

The temperature of culture, which was not regulated at all in all the experiments, varied in a wide range both diurnally and seasonally. The highest and lowest temperatures attained were 38.0°C in August (afternoon on fair days) and −3.0°C in January (before sunrise). Early in the morning in January and February, the surface of culture was often covered with ice with many small holes or humps with holes which were formed by air bubbles

blown from the aeration pipes (Fig. 7). After sunrise, however, the ice melted sooner or later, and in the afternoon of fair days, which are very frequent in winter in Tokyo, the temperature of culture solution used to rise up to about 10°C.

Since the cultures were open to atmosphere, the volume and concentration of culture solution fluctuated owing to evaporation and rain or snow. The volume of culture solution was maintained as constant as possible, by adding water after continued dry days, or by removing a part of culture solution (and harvesting the algal cells) on or after rainy and snowy days. It has been confirmed that with the culture medium used, change in concentration of nutrient salts in a range of 1/2~2.5-fold of the normal value did not affect appreciably the growth rate of algae. The pH value of the culture solution tended to increase with the progress of culture. It was regulated in the range between 5.4 and 6.4 by adding occasionally inorganic acids (sulfuric, nitric or phosphoric) to the solution. While some cultures could be continued for more than 2 months — with occasional harvesting of cells and returning the culture medium to the pond — some cultures had to be discontinued (especially in warm seasons) within 10 days or 2 weeks. The main reason of this discontinuance was the contamination by other microorganisms, such as *Protozoa* and *Rotifera*, which appeared frequently during the period from June to November*.

The results obtained during the period from August 1954 to June 1955 are summarized in Table 1, in which the average, maximum and minimum daily yields obtained with different algal strains are given together with the data of average atmospheric temperature and the average solar energy (in terms of gcal/cm^2-day) in each month. The figures in parentheses given in the 3rd, 4th and 5th columns are the number of days, during which the recorded yields were observed.

As may be seen from the table, the highest yield obtained with thermophilic strains was as much as 22–28 g/m^2-day, although it lasted only for several days on fair days of June, August and October. On the other hand, the highest average yield obtained in the coldest month of the year (January) was 2.0–2.8 g/m^2-day (with *Chlorella ellipsoidea*), that is, about 1/10 of the highest yield shown by thermophilic strains in warmer season.

These experiments have shown that the new type of the open culture method — when algal strains of different temperature-tolerance are used according to the season, and at least under climatic conditions such as found in Japan — is not only usable throughout the year, but also gives

* Towards the end of the present series of experiments we found that 2, 4-dinitro-6-cyclohexyl phenyl acetate and some other drugs are effective in killing *Protozoa* and *Rotifera* without affecting the growth of algae. The effect of these drugs were not known to us in the summer of 1954 when most of the contamination troubles occurred in our experiments. Details on the drugs for preventing the contamination will be reported in a subsequent paper.

Table 2. Growth of various strains of *Chlorella* and *Scenedesmus* in the open bubbling culture in various seasons of the year.

Among the algal strains used, Ctm37, Ctn1, Ctn6, Ctn7 and Stm32 were temperature-tolerant.

The figures for the average intensity of solar radiation (including infrared light) are those measured by the Central Meteorological Observatory, Tokyo, using the Robitzsch actinograph.

Compared with the atmospheric temperatures given in the table, the average temperatures of culture solution used to be more or less higher. The highest and lowest temperature attained in the culture solution were 38°C (in August) and −3°C (in January), respectively.

Algae	Month	Yield in g/m²-day			Average atmospheric temp. (C)	Average daily quantity of solar radiation (gcal/cm²-day)
		Average (days)	Maximum (days)	Minimum (days)		
Chlorella Ctm37	Aug.	16.1 (11)	21.1 (7)	7.3 (4)	27.0	282
	Sept.	12.3 (17)	15.5 (8)	4.4 (3)	24.6	255
Scenedesmus Stm32	Oct.	9.5 (14)	13.2 (7)	3.2 (5)	15.5	173
	Oct.	7.6 (12)	11.3 (5)	2.8 (3)	15.5	173
	Nov.	7.4 (15)	11.0 (7)	0.2 (3)	11.7	169
Chlorella Ctn1	Oct.	12.8 (31)	21.8 (8)	5.0 (14)	15.5	173
	Nov.	6.2 (9)	—	—	11.7	169
Scenedesmus Sm36	Nov.	8.4 (17)	13.0 (6)	2.8 (5)	11.7	169
	Dec.	3.8 (13)	4.5 (5)	3.1 (5)	7.1	146
Chlorella ellipsoidea	Nov.	10.6 (17)	13.2 (9)	5.3 (4)	11.7	169
	Dec.	5.8 (31)	8.8 (9)	0.2 (6)	7.1	146
	Jan.	2.0 (31)	—	—	3.8	183
	Feb.	6.4 (7)	9.1 (2)	4.0 (2)	6.2	
	Dec.	2.8 (10)	3.5 (8)	0.2 (2)	7.1	146
	Jan.	1.4 (13)	2.2 (8)	0.1 (4)	3.8	183
	Dec.	5.8 (16)	7.1 (13)	0.2 (3)	7.1	146
	Jan.	2.8 (31)	3.3 (26)	0.1 (3)	3.8	183
	Feb.	7.6 (19)	9.3 (7)	6.1 (3)	6.2	215
Chlorella Cn2	Mar.	4.3 (15)	6.3 (6)	1.1 (2)	8.7	200
Chlorella Cn3	Apr.	8.8 (15)	15.3 (4)	3.1 (2)	13.6	265
Chlorella Cn4	Apr.	10.8 (12)	18.3 (3)	3.0 (4)	13.6	265
Chlorella Cn6	May	12.9 (21)	20.4 (4)	5.8 (2)	18.2	320
	June	16.8 (27)	28.0 (3)	5.0 (2)	22.7	323
Chlorella Cn7	June	13.2 (14)	25.0 (2)	4.4 (2)	22.7	323

better yields than the other methods thus far attempted for outdoor mass-culturing of algae. The yields reported above are those obtained without regulation of culture temperature throughout the year. The fact that we can dispense with the equipment for temperature regulation is a great advantage of the open culture system over the closed culture system, in which the temperature rises up to 50°C in summer time without regulation. The simplicity and durability of construction are also advantages of our open system compared with the closed circulation system.

It should be mentioned, however, that the open culture method has disadvantages in the following points:

(1) An enormously large quantity of CO_2-enriched air is required for aeration, and a considerable part of CO_2 is wasted without being utilized by algal cells.*

(2) To keep the culture clean and to lessen the possibility of contamination, occasional removal of dust is necessary.**

(3) The water content in the culture must be regulated from time to time, although the fluctuation of water content in the range of 1/2~2.5-fold does not appreciably affect the growth of algae.

(4) Even when the culture is well aerated, the algal cells often tend to precipitate or aggregate, which causes troubles in operating the culture. The tendency of precipitation and aggregation of algal cells differs widely according to the algal strain. In the worst case it cannot be eliminated even when the aeration rate is raised to as much as 500 ml per minute per liter of culture solution.

SUMMARY

(1) Using a new type of culture ponds ("open bubbling sytem") according to the principle of Gummert *et al.*, various strains of unicellular algae were grown outdoors without artificial regulation of culture temperature.

(2) It was found that, with adequate algal strains, an average daily yield of 16–17 g/m^2-day (maximum 22–28 g/m^2-day) was obtainable in summer time, and 2–2.8 g/m^2-day (maximum 3.3 g/m^2-day) in winter time. The highest and lowest yield were obtained in August and January, respectively.

(3) The advantages and disadvantages of the culture method used, as compared with the other methods thus far proposed for outdoor mass-culturing of algae, were discussed in detail.

* The quantity of CO_2-air needed for running the open culture system is estimated to be as much as 5–10 times that required for operating the "closed circulation system" such as devised by Arthur D. Little, Inc. (2).

** In our open ponds operated in the city of Tokyo, cleaning at an interval of about 2 months was necessary.

This work was carried out as a part of the program directed by Professor Hiroshi Tamiya. We are indebted to the Ministry of Education, the Rockefeller Foundation, the Ministry of International Trade and Industry, and the Ministry of Agriculture and Forestry for grants which enabled the research to be carried out.

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SEASONAL VARIATION OF GROWTH RATE OF VARIOUS STRAINS OF UNICELLULAR ALGAE UNDER NATURAL LIGHT- AND TEMPERATURE-CONDITIONS

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In the project of mass-culturing of algae, the selection of fast growing strains of algae is a matter of paramount importance. Since different algal strains are differently adapted to light- and temperature-conditions, the suitableness of algae to be mass-cultured will vary according to the climates or seasons. It is, therefore, important to investigate in what manner the growth activity of each algal strain changes with the variation of natural environmental conditions. In their kinetic studies on the growth of *Chlorella*, Tamiya *et al.* (1) have proposed a formula, by which the growth rate under natural conditions (with sufficient provision of nutrients and carbon dioxide) may be assessed from the data obtained by some simple culture experiments in the laboratory. Since, however, the natural light- and temperature-conditions are by far more complicated than were assumed in the theory of Tamiya *et al.*, their formula can give, at best, only a rough estimation of the algal yield which would be obtainable under natural field conditions.

To obtain a straightforward informations about the seasonal variation of growth rate shown by various algal strains, we have devised a simple method of outdoor culturing, and thereby followed the growth rates throughout the year. The data obtained by this method were found to reproduce fairly well the actual yield obtained from larger-scale outdoor cultures; thus the method has proved to be usuable for testing the suitability of algal strains as materials of mass-culturing in different climates or seasons. Described herein are the method used and the main results obtained.

METHOD

The oblong flat flasks (with a uniform inner thickness of 3 cm and a total capacity of 600 ml) which were described in detail in our earlier papers (1,2) were used as containers of culture. The flasks were filled with 250 ml of culture medium of the following composition, per liter:

3.0 g Urea, 2.5 g $MgSO_4 \cdot 7H_2O$, 1.25 g KH_2PO_4, 55 mg $CaCl_2 \cdot 2H_2O$, 21 mg $ZnCl_2$, 2 mg $CoCl_2 \cdot 6H_2O$, 8 mg $MnSO_4 \cdot 5H_2O$, 8 mg $CuSO_4 \cdot 5H_2O$, 88 mg $Na_2B_4O_7 \cdot 10H_2O$, 3.7 mg $3(NH_4)_2O \cdot 7MoO_3 \cdot 4H_2O$, 25 mg $FeSO_4 \cdot 7H_2O$, 37 mg Ethylenediamine tetraacetic acid (di-sodium salt).

After autoclaving and inoculation of algal cells, the flasks were fixed, at an angle of 45° (directed towards south), on a wooden bar which was placed horizontally across the edges of a trough (see Fig. 1). The

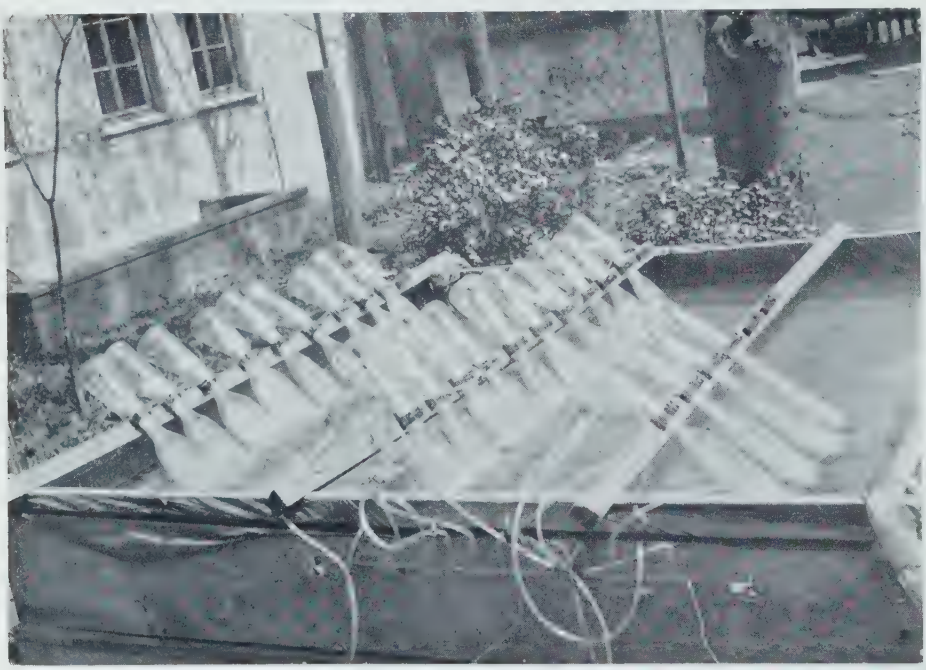

Fig. 1. Outdoor cultures using flat flasks which were placed at an angle of 45°, being partly dipped in shallow stagnant water, whose temperature was left unregulated throughout the year.

trough was 30 cm in depth and contained water of 15 cm in depth, so that the portion of the flasks filled with the culture solution was dipped into the water. The water in the trough was kept stagnant, so that its temperature varied both diurnally and seasonally in the manner as it approximately reproduced the temperature variation occurring in the large-scale mass culture of "open system" (3).* Considering that the large scale algal culture are ordinarily run at high population densities, the initial concentration of algae in the culture was made 5 or more ml packed cell volume per liter. It has already been shown that in such a high population density the growth rate becomes proportional to the area of illuminated surface (1). When the population density surpassed the level of about 20 ml per liter, the density was lowered to the initial level.

* In warmer seasons, the trough water was desinfected with 0.1 percent sublimate in order to prevent the growth of bacteria which would render the water turbid.

The culture was constantly aerated with air containing 3–5 percent carbon dioxide at a rate of 200 to 300 ml per minute. To protect the opening of the culture flask from rain and dust, the top of the flask was covered with a conical beaker as shown in the figure. During the whole period of experiments the conditions other than the temperature and light were kept optimum by refreshing the culture medium at an interval of 6 days, and, if necessary, also by replacing the algae with fresh cells of the same strain.

The growth of algae was followed by measuring once a week the concentration of cells in the culture, and taking into consideration the volume and the area of illuminated surface of each culture, the growth rates were calculated and compared in terms of the increase of dry weight (gram) per square meter per day.

RESULTS AND DISCUSSION

The results of experiments run during the period from February 1954 to January 1955 are summarized in Table 1. The figures given are the averages of daily yields which were determined 4–5 times in each month. Of the six strains tested, *Chlorella* Ctm37, Ctm16, Tx71105* (4), and *Scenedesmus* Stm32 were temperature-tolerant strains, while the others were of ordinary temperature tolerance ("mesophilic").

Table 1. Average growth rate (g/m^2-day) in each month shown by different algal strains in the test cultures.

Month	Average daily yield (g/m^2-day) shown by					
	thermophilic				mesophilic	
	Chlorella Ctm37	*Chlorella* Ctm16	*Chlorella* Tx71105	*Scenedesmus* Stm32	*Chlorella ellipsoidea*	*Scenedesmus* Sm36
1954, Feb.	0	0	0	0.4	6.3	0.9
Mar.	1.0	0.4	0	2.5	12.3	3.8
Apr.	5.1	5.3	3.6	5.4	17.6	5.4
May	6.6	7.9	11.6	—	17.5	9.0
June	6.5	9.2	10.0	—	15.5	10.5
July	10.3	8.9	11.1	—	12.8	11.0
Aug.	12.1	10.3	11.2	7.8	7.2	—
Sept.	12.5	16.8	10.4	10.8	12.2	16.0
Oct.	12.3	15.6	9.8	7.3	16.5	9.6
Nov.	3.8	6.3	4.8	5.9	16.3	9.9
Dec.	0	0	0.2	2.0	6.1	2.8
1955, Jan.	0	0	0	0	4.4	0

As may be seen from the table, different strains showed widely different growth activity according to the season. Of the 6 strains tested, it was only *Chlorella ellipsoidea* that could grow positively in all months through-

* This strain was kindly supplied by Dr. Jack Myers, to whom we take this opportunity of extending our thanks.

out the year. All the thermophilic strains showed no or almost no growth in winter months, December, January and February. Sensitivity to cold was most pronounced in the Texas-strain (Tx 71105) which did not grow even in March. While the thermophilic strains grew well in summer months, *Chlorella ellipsoidea* grew relatively poorly in summer months, especially in August which is the hottest period of the year in Japan.

In Table 2 are given the data of temperature and solar radiation meas ured during the period of the experiment. The maximum and minimum water-temperatures given are those attained in the stagnant water in the trough, and therefore, represent the values attained by the algal suspensions contained in the culture flasks. The amounts of solar radiation given in the table are those measured by the Central Meteorological Observatory, Tokyo, using a Robitzsch actinograph. They represent the quantity of whole sky radiation (including infrared) received by a horizontal surface.

Table 2. Temperature and solar radiation in each month during the experiment.

Month	Average atmospheric temperature (C)	Water-temperature (C)		Average solar radiation in gcal/cm²-day
		max.	min.	
1954, Feb.	5.6	12	−2	203
Mar.	8.4	18	1	253
Apr.	14.8	21	6	255
May	17.6	23	11	269
June	18.3	25	12	200
July	22.3	29	17	232
Aug.	27.0	35	20	282
Sept.	24.6	30	16	255
Oct.	15.5	26	10	173
Nov.	11.7	19	6	169
Dec.	7.1	12	1	146
1955, Jan.	3.8	11	−3	183

Characteristic features in seasonal variation of growth rate shown by mesophilic and thermophilic strains are apparent from the curves in Fig. 2, in which the results obtained with *Chlorella ellipsoidea* (mesophilic) and *Chlorella* Ctm37 (thermophilic) are shown together with the data of average atmospheric temperature and the average solar radiation observed in each month.

Taking into consideration that the energy content of algal cells is 5.5 kcal per gram (5), we can calculate the efficiency of solar energy conversion shown by each algal strain. The monthly variation of the efficiency (on a total radiation basis) observed with *Chlorella ellipsoidea* and *Chlorella* Ctm37 is illustrated in Fig. 3. In the case of *Chlorella ellipsoidea* the efficiency was highest in October and November (5.3 percent) and lowest in January and August (1.3–1.4 percent).

In parallel with the test cultures described above, large-scale outdoor cultures of the "open bubbling system", about which we have reported in

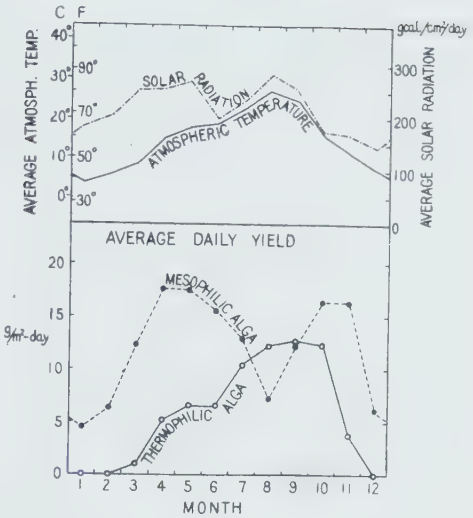

Fig. 2. Monthly variations of average solar energy, atmospheric temperature, and the average daily yield obtained with *Chlorella ellipsoidea* (mesophilic) and *Chlorella* Ctm37 (thermophilic).

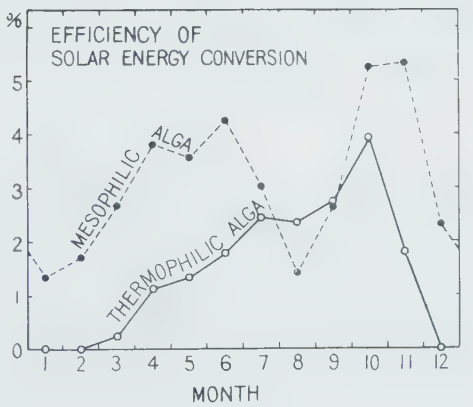

Fig. 3. Monthly variation of the efficiency of solar energy conversion effected by *Chlorella ellipsoidea* and *Chlorella* Ctm37. Efficiency was calculated on a total radiation basis and the energy content of algal cells was considered to be 5.5 kcal per gram.

the preceding paper (3), were run using some of the strains listed in Table 1. In Table 3 the yield obtained from these cultures are compared with the data given in Table 1. It may be seen that the results of our test cultures coincided fairly well with those obtained in actual operation of open outdoor cultures. From the data presented in Fig. 2 it is inferred that if we use *Chlorella ellipsoidea* and *Chlorella* Ctm37 adequately according to the season, we can obtain an average of 12.4 g dried algal cells per square meter per day throughout the year. It may, therefore, be deduced that by operating the open outdoor cultures adequately using these two strains we can obtain 18 metric tons of dried *Chlorella* per acre per year.

Table 3. Comparison of the yields (g/m²-day) in the test cultures with those obtained from large-scale open outdoor cultures.

Month	Culture	Yield in g/m²-day of			
		Chlorella Ctm37	*Scenedesmus* Stm32	*Chlorella ellipsoidea*	*Scenedesmus* Sm36
Jan.	Test	0	0	4.4	—
	Open	0	0	1.4–2.8	
Feb.	Test	0	0.4	6.3	—
	Open	0	0	6.4–7.6	
Aug.	Test	12.1	—	—	—
	Open	16.1			
Sept.	Test	—	10.8	—	—
	Open		12.3		
Oct.	Test	—	7.3	—	—
	Open		7.6–9.5		
Nov.	Test	—	5.9	16.3	9.9
	Open		7.4	10.6	8.4
Dec.	Test	0	—	6.1	2.8
	Open	0		5.8	3.8

SUMMARY

(1) Using a simple method of test cultures, seasonal variation of growth rate of various strains of unicellular algae under natural light- and temperature-conditions was investigated.

(2) The strains of ordinary temperature tolerance ("mesophilic"), such as *Chlorella ellipsoidea*, showed positive growth in all months throughout the year, although their growth was considerably suppressed in the hottest month, August. In contrast, the thermophilic strains grew actively in summer months, but showed no growth in winter months, December, January and February.

(3) Taking into consideration the quantity of solar radiation and the energy content of algal cells, the efficiency of solar energy conversion shown by some algal strains was calculated. In the case of *Chlorella ellipsoidea* the efficiency (on a total radiation basis) was highest in October and November (5.3 percent) and lowest in January and August (1.3–1.4 percent).

(4) It was shown that the growth rate of algae determined by the test cultures agreed fairly well with the actual yield obtained from the open outdoor cultures in each season. It was inferred that from large-scale outdoor cultures using mesophilic and thermophilic strains adequately according to the season, a yield of 18 metric tons (dry weight) of algae may be obtained per acre per year.

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EFFECT OF VARIOUS CHEMICALS UPON THE GROWTH OF *DAPHNIDS*

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In the previous paper[1], *Tolypothrix tenuis* (from Borneo) was proved to be the most powerful nitrogen-fixing blue-green alga which was isolated from 643 samples collected from rice fields in various districts of the Far East and the South Seas. The amount of nitrogen fixed in 2 months was 9.6 mg in Erlenmeyer flasks containing 100 ml. of liquid medium.

The effect of this alga on the growth of rice plants was examined in both pot-culture and paddy fields. In pot cultures the leaf length of the rice plants increased on an average by 17 per cent and the number of ears increased by 30 per cent when *Tolypothrix* was present. In the paddy fields, the increase in the rice yield was about 20 per cent, according to the average of results of nine agricultural experiment stations and two universities.

But occasionally there occured a serious problem in that the seeded algae completely disappeared from the field within 1 or 2 weeks. In such cases, almost without exception, the field water was abundant with daphnids which eventually consumed the seeded algae entirely. Accordingly, some measure was sought which would prevent the increase of the population of daphnids. Two plans were drawn up to suppress the growth of these water-fleas; the first was to look for some transmissible diseases of daphnids, and the second was to search for chemicals which would kill the daphnids in extremely low concentrations.

With regard to daphnid diseases, research was done on about 800 soil samples, but no satisfactory results were obtained. The present paper deals with the poisonous chemicals tested, some of which are both inexpensive and effective in extremely low concentrations.

EXPERIMENTAL

Recently[2], a convenient method was reported for growing large populations of daphnids by feeding mixtures of *Chlorella* and *Tolypothrix*. The experiments reported in this paper were performed with a modification of the above-mentioned method: the culture of daphnids was made in a series of Erlenmeyer flasks (300 ml capacity) each containing 100 ml of filtered pond water as a culture medium. For feeding the daphnids half dried Kanumatsuchi (a volcanic ash) was mixed with *Tolypothrix tenuis* and

Table 1. Effect of various agricultural chemicals on daphnids and blue-green algae

Drugs	Main chemical components	Minimum lethal concentrations for *Moina macropa* (ppm)	Maximum innocuous concentrations for *Tolypothrix tenuis* (ppm)
Parathion*	Diethyl-p-nitrophenylthio-phosphate 47%	0.00075	1
Folidol*	〃 46.6%	0.001	10
Derris emulsion	Rotenone 2%	0.001	0.1
Malathon*	0, 0-Dimethyl dithiophosphate of diethyl mercapto succinate	0.01	5
Furethrin*	Pyrethrin	0.25	0.1
Chlordane*	1, 2, 4, 5, 6, 7, 8, 8-Octachloro-4, 7-methano-3a, 4, 7, 7a-tetrahydro-indane	1	1
Dieldrin*	1, 2, 3, 4, 10, 10-Hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8a-octahydro-1, 4, 5, 8-dimethanonaphthalene	1	1
Septol*	Benzalkonium chloride 10%	1	1
Isiguro 40*	Nicotine sulphate 40%	1	100
DN	2, 4-Dinitrocyclohexyl phenol	1	1
PCP	Pentachlorophenol	1	1
PCP Na	Pentachlorophenol Na-salt	1	10
Nikkarin T*	Tetraethyl pyrophosphate 35% Hexaethyl tetraphosphate 15%	2	100
DDT (20)	Dichloro diphenyl trichloroethane 20%	2	10
Copper sulfate	$CuSO_4$	2	1
Potassium cyanide	KCN	2	1
DNA	2, 4-Dinitrocyclohexyl phenyl acetate	10	1
γ-BHC	γ-Benzenehexachloride	10	2
Kativ*	Vitamin K_3 sodium bisulfite 2%	50	10
p-Oxybenzoic acid butyl ester	p-Hydroxybenzoic acid buthyl ester	50	1
Mikezol*	o-Dichlorobenzene 49%	100	10
Neo Gikuron*	Ethylene dibromide 9.55% p-Dichlorobenzene 10.56%	500	100

* Trade names

Chlorella ellipsoidea in proportion of 9 parts ash to 1 part algal mixture. Chemicals to be tested were put into flasks in various concentrations and a definite number of *Moina macropa* were inoculated together with 0.3 g Kanumatsuchi; the flasks were placed at room temperature (23°–28°C) with diffuse day light, and the growth of *Moina* was followed by counting the number of individuals in each flask. Observations were made on the minimum lethal concentrations of chemicals after twenty-four hours. To determine the influence of the test chemicals on the blue-green alga, 0.05 g (fresh weight) of *Tolypothrix tenuis* was put in test tubes with 10 ml tap water, and exposed under day light in a green-house (30°–34°C).

In five days the algae grew, forming a film over the surface of water, and some algal bodies climbed several millimeters up the tube wall. After certain chemicals were put into tap water in sufficient concentrations, the algae bleached and failed to form the film or to climb along the test tube. The maximum innocuous concentrations of chemicals upon the growth of blue-green algae, were determined by comparing film-formation and climbing with those of the controls.

In Table 1 are shown the minimum lethal concentrations of some chemicals for *Moina macropa* and the maximum innocuous concentration for the growth of *Tolypothrix tenuis*. Parathion and Folidol, which contain diethyl p-nitrophenolthiophosphate, are decisively effective as daphnidkillers even in the lowest concentration applied, and Derris emulsion, Malathon, Furethrin etc. follow in that order of effectiveness. On the other hand, the maximum innocuous concentration to blue-green algae was found to be

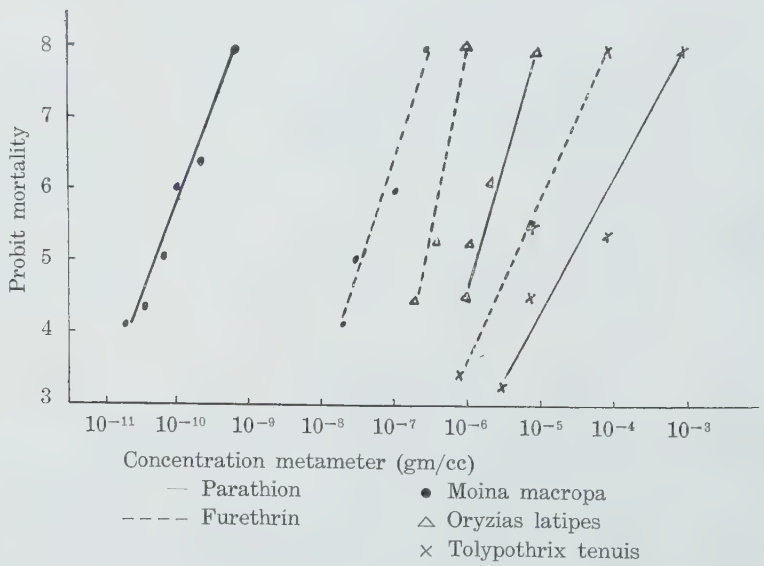

Fig. 1. Probit mortality-concentration curves showing the action of Parathion and Furethrin upon three organisms.

1 ppm of Parathion and 10 ppm of Folidol. These concentrations are about one thousand times the minimum lethal concentration to daphnids and this fact indicates that Parathion and Folidol are the most effective compounds for killing daphnids without affecting the growth of blue-green algae. On the other hand, some chemicals such as Furethrin, DNA, γ-BHC, Kativ etc. are more noxious to blue-green algae than to daphnids.

Further studies were made to determine the LD50 of Parathion and Furethrin against *Moina macropa*, *Oryzias latipes* (a fresh water fish) and *Tolypothrix tenuis* by calculating probits (Fig. 1). Parathion was proved to be considerably more noxious to daphnids than to fresh water fish or to algae.

SUMMARY AND DISCUSSION

Twenty two chemicals were tested for their effectiveness in killing daphnids, and at the same time their maximum innocuous concentrations towards blue green algae were determined. It was observed that diethyl-p-nitrophenylthiophosphate is most effective as a daphnidkiller, and is, at the same time, innocuous for the growth of blue-green algae.

For several years Parathion or Folidol, which is innocuous for the rice plant in a concentration of 250 ppm, has been used as an insecticide in rice fields, and now it is also shown to kill daphnids in a concentration of 0.00075 ppm. Assuming that the depth of the water in a paddy field is 10 cm, the volume of water is 400,000 liters per acre. If the diluted solution of only 20 ml Parathion is sprayed on this field, the concentration of the drug in the water of the field will become 0.05 ppm. This concentration is about one hundred times the minimum lethal concentration of daphnids, and therefore this drug is remarkably efficient.

It should be remembered, on the other hand, that daphnids are known to be a favourite food for fresh-water fish. If daphnids are killed by spraying of the drug on the paddy field sooner or later it may affect the growth of fresh-water fish in the paddy field water by the continued scarcity of daphnids.

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STUDIES ON *ASPERGILLUS USAMII MUT. SHIRO-USAMII* AND ITS INDUSTRIAL APPLICATION*.

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INTRODUCTION

Mutations have been artificially induced in the industrially useful fungus *Aspergillus usamii* in order to obtain improved strains. We have already reported[1] on the mutations resulting from ultra-violet irradiation and the morphology of the mutants; we classified 81 newly obtained mutants, which were obviously different from the original strain, into 13 types. Mutants beloning to the blackish brown type (Saccardo's Umber XXIX or Medal Bronze IV), the brown type (Tawny Olive XXIX) and the tan type (Light Pinkish Cinnamon XXIX) were found to differ morphologically only in the color of their conidial heads. We also recognized a constant tendency among these mutants to change in one direction; that is, from the black color of the original strain the colors of the conidial heads became lighter and lighter with continued treatment.

This report will describe the heterocaryosis in these mutants, as well as physiological characteristics relating to their application to the saccharification of mashes for alcoholic fermentation. We found their amylolytic and acid producing powers almost as same as those of the original strain, or in some cases better. Therefore we decided to test a tan type, whose conidial head color was the lightest, for industrial alcoholic fermentation.

EXPERIMENTS

1. Description of Morphological Characteristics.

We used following mutants as test organisms: B403, B406, and B407, a tan type described in the previous report[1], B207, and B604, a brown type, and B607, a blackish brown type, and as controls we used the original *Aspergillus usamii* R-0635[2], *Aspergillus awamori* 22-1[3] and *Aspergillus kawachii* A431[4]. Their giant colonies were formed and observed on Czapeck's agar, Koji extract agar and nitrite medium at 30°C respectively. The description of a tan type mutant, named *Aspergillus usamii mut. shiro-usamii*, follows.

* This was read at the Anual Meeting of the Agricultural Chemical Society of Japan in April, 1952.

Description of Aspergillus usamii mut. shiro-usamii

The morphological and cultural characteristics of the mutant are the same as those of the original strain except for the color of conidial heads. Colonies grow on Czapeck's agar, attaining a diameter of 6 cm in three days at 30°C and having light yellow hyphae. They also grow rapidly on Koji extract agar and nitrite medium. Conidial heads Pinkish Buff XXIX (Ridgway) in color, globose or radiate, usually 250 to 500 μ in diameter. Conidiophores mostly 3 to 5 mm in length, 15 to 25 μ in diameter, walls smooth. Vesicles globose, 50 to 70 μ in diameter. Sterigmata in two series; primaries 11 to 20 μ by 5 to 6 μ, secondaries 7 to 9 μ by 3 to 4 μ. Conidia globose, mostly 3.5 μ loosely rough.

2. Ultra-violet Ray Irradiation of Ultra-violet Induced Mutants.

Not only *Aspergillus usamii mut. shiro-usamii* belonged to tan type, but the brown type mutants and the blackish brown are stable strains as observed in their successive cultures or mono-spore cultures. These mutants were irradiated further by ultra-violet light. The experimental method has been given in the previous report[1], in which some of the results involving the tan type mutant were also reported.

Mutants belonged to pigmentless, small heads, restricted, wet, sterile types were obtained from these three types respectively. A Pale Ochraceous Buff XV mutant was obtained from the tan type. As the conidial head color is lighter than the parent we classify this as an albino type. In this experiment no strain in which the conidial head color was like that of the original *Aspergillus usamii* was obtained. No back mutation was observed and no strain showing darker conidial head color as those of three types mutants was obtained by irradiation with ultra-violet.

3. Formation of Hetrocaryotic Heads.

It is observed that several characteristics other than the color of conidial heads are almost same in the original *Aspergillus usamii* and in mutants of the blackish brown, brown and tan types. Therefore we tried to induce heterocaryosis among these similar strains. The strains used are as follows: *Aspergillus usamii* R-0635, with its mutants, blackish brown type B607, Brown type B207 and B604, and tan type B403, B406 and B407, *A. awamori*[3] 22–1, *A. aureus*[3] 22–7, *A. inuii* R–0436[2], *A. kawachii* A431[4], *A. niger* 2126, *A. cinnamomeus* NRRL 361, *A. terreus* 2613, *A. candidus* 26–3, *A. wentii* 32–6, *A. tamarii* 53–6, *A. oryzae* A, *A. sojae* SH 10–1, and *A. ochraceus* 24–6. These include the typical head colors of Aspergillus strains.

Experimental method:

All possible combinations of pairs were made with the strains listed above by inoculating on Czapeck's agar and Koji extract agar on Petri plates,

making parallel lines 2 cm apart. The results showed that in each pair both strains anastomosed where they met. Subsequently conidial heads appeared along the interface. Microscopic examination of the interface showed that heterocaryotic heads were not formed on Czapeck's agar, though we observed them clearly on Koji extract agar after 10 days.

(A) In the case of the combination between tan type B406 and brown type B604 or tan type B403 and brown type B207 or tan type B403 and *A. usamii* R-0635 or brown type B407 and *A. usamii* R-0635, conidial heads along the interface between the two colony areas were observed to be composed of conidial chains having both colors. For instance, in the case of the tan and brown types, we find heads, composed of the tan conidial chains of B406 and the brown of B604 (Fig. 1).

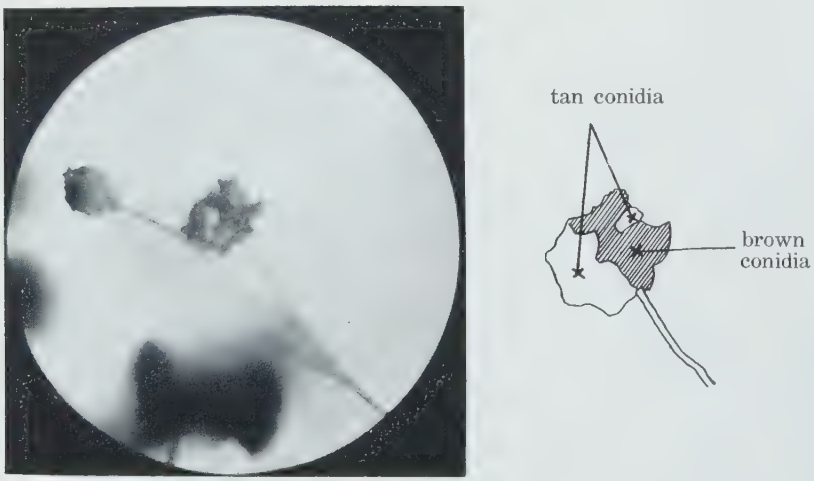

Fig. 1. Heterocaryotic head of tan type mutant and brown type mutant of *Aspergillus usamii*, showing chains of tan and brown conidia in one conidial head.

(B) Along the interface of tan type B406 and brown type B604, black conidial heads which are the same as those of the original strain, *Aspergillus usamii*, develop.

(C) None of the above phenomena were observed in combinations among many other species and strains. It was not easy to distinguish conidial heads which were similar in color, as culture conditions which are apt to induce anastomosis may cause confusion mix the types too closely. Pontecorvo (1946)[5] describes that heterocaryotic heads having brown conidial chains and light colored conidial chains are formed between *Aspergillus tamarii* and its light colored mutant. In the studies of heterocaryosis between *Aspergillus fonsecaeus* mutants, Raper and others report[6] that black colored heterocaryotic heads resembling those of the original strain are obtained.

4. Amylolytic and Acid Producing Powers.

The important physiological characteristics of *Aspergillus usamii mut. shiro-usamii*, that is, the amylolytic and acid producing powers, were compared with those of *Aspergillus usamii* R-0635, *Aspergillus awamori* 22-1 and *Aspergillus kawachii* A431.

(A) Dry sweet potato slice medium

Experimental method: 3 g of dry sweet potato slices are milled, put in a 100 cc Ehrenmeyer flask with 1.5 cc of distilled water, soaked for 15 hours and cooked for an hour. A previous culture, grown at 30°C for 7 days on Koji extract agar, is inoculated by plantinum loop into the above and cultivated at 30°C for 72 hours. During the culture period the sample is shaken well once every 24 hours in order to mix the mycelium as thoroughly as possible. After this period, 2 cc of distilled water are added and the saccharifying process is done for 3 hours. The filtrate obtained after this process to 100 cc with distilled water. The starch decomposing power is obtained by determing the reducing sugar by iodometry in a 5 cc sample. Another 5 cc sample is used for calculating the acid producing power by titration with N/10 NaOH. The results, as shown on Table 1, indicate that the 3 strains of *Aspergillus suamii mut. shiro-usamii* are superior in acid producing power, compared with the original strain, but are inferior in amylolytic power.

Table 1. Amylolytic power and acidity on sweet potato medium.

Strain	*A. usamii*	*A. usamii mut. shiro-usamii*			*A. awamori*	*A. kawachii*
	R-0635	B406	B407	B403	22-1	A431
Amylolytic power %	40.9	35.4	37.8	36.9	42.2	40.2
Acidity N/10 NaOH	3.4	4.4	4.0	5.3	0.5	0.7

(B) Bran medium

Experimental method; 8 g of bran and 2 g of rice hulls are put in a Ehrlenmeyer flask with 3.5 cc of distilled water, stirred well, and sterilized after an hour. A platinum loop volume of a previous culture grown at 30°C for 7 days, is inoculated into this medium and cultivated at 30°C for 66 hours. 100 cc of 1% NaCl solution are then added to the finished rice hull Koji medium, kept at 37°C for 3 hours, and filtered; in this way an enzyme solution is obtained. Then 100 cc of Michaelis buffer solution (PH 4.8), 10 cc of the above enzyme solution and 1 cc of toluene are added to 100 cc of 2% soluble starch solution and kept at 40°C. After 1.5 and 3 hours, the amount of starch decomposed power is obtained by determining the reducing sugar by iodometry[7]. The results are given in Table 2. The amylolytic power of three strains of *Aspergillus usamii mut. shiro-usamii* is stronger than that of the original and two other control strains.

Table 2. Amylolytic power on bran medium.

Period of enzyme action \ Strain name	*A. usamii*	*A. usamii mut. shiro-usamii*			*A. awamori*	*A. kawachii*
	R–0635	B406	B407	B403	22–1	A431
1.5 hrs.	46.0%	51.5	52.3	56.6	30.5	30.5
3.0 hrs.	53.0	61.1	61.5	61.8	37.9	40.0

(C) Amylase activity and saccharification.

Experimental method; 200 cc of 1% NaCl solution are added to 10 g of bran Koji and kept at 37°C for 3 hours. Its filtrate is used as the enzyme solution. It is added to a starch solution and 2 cc is sampled (at regular intervals) to calculate, by determining the reducing power of each sample, the percentage of hydrolyzed glucosidic bonds. Saccharification curves plotted from the above results are not so very different among strains of *Aspergillus usamii mut. shiro-usamii*. Therefore their values are shown on Table 3, but the curves will not be shown here.

Table 3. Amylase activities* of *Asp. usamii* and *Asp. usamii mut. shiro-usamii*.

Period of enzyme action \ Strain name	*A. usamii*	*A. usamii mut. shiro-usamii*		
	R–0635	B406	B407	B403
45 min.	17.7%	9.7%	14.0%	15.5%
90 ,,	31.4	26.9	28.5	—
165 ,,	47.7	42.6	43.9	42.1
4.5 hrs.	54.6	52.7	49.8	51.9
6 ,,	59.1	58.9	54.7	56.2
8 ,,	59.6	58.7	59.6	59.3
24 ,,	64.3	65.9	65.8	64.8
Achromatic point, of iodine-starch reaction	120 min.	165 min.	165 min.	165 min.

* Amylase activities are indicated in terms of percentage of glucosidic bonds hydrolyzed.

5. *Industrial Application.*

It might be beneficial to use the light colored mutant for the alcoholic fermentation industry instead of present black colored *Aspergillus usamii*, judging from several characters, such as starch decomposing power, as described above. Practical tests have demonstrated the advantages. Therefore, we will describe here a new type of alcoholic fermentation in an open system by the Koji method, using *Aspergillus usamii mut. shiro-usamii*

B406 as a test strain. This was done in Aug. 1951. The manufacturing of the seed Koji for inoculum, the operations and conditions for manufacturing the Koji, and other factors are the same as for the original strain. However, for the manufacturing Koji, a lower temperature than the general Kuro-Koji method is required. Table 4 shows the course of fermentation in a tank

Table 4. Course of fermentation.

Hr.	Total reducing sugar %	Alcohol %	Direct reducing sugar %	Acidity N/10 NaOH cc per 10 cc	Bllg.	Temp. °C
0	9.13	0.7	1.85	0.8	6.8	26.5
3.5	—	1.2	1.47	0.9	4.7	28.3
10.5	—	2.2	1.75	1.1	3.3	30.5
16.5	—	3.5	—	1.3	1.3	33.5
19.5	—	3.7	1.24	1.4	0.9	34.5
24.5	—	4.5	0.72	1.4	0.0	35.0
34.5	—	5.4	0.50	2.1	−1.0	36.0
40.5	—	5.5	—	2.3	−1.5	37.5
47.5	—	5.7	0.45	2.7	−2.0	36.5
58.5	1.00	5.9	0.37	3.8	−2.0	36.0

holding about 1, 190 gal. when crude starch (in Japanese, Doniku Dempun) is used for the chief material. We find that the volume of alcohol production during the first period of fermentation is rather less, compared with *Aspergillus oryzae.* But this volume increases rapidly in the later part of the fermentation period and remains high until just before the distillation process. This is also characteristics of the original strain.

CONCLUSION

1. Studies were made concerning the morphology and classification of a strain whose conidial head color was light pinkish cinnamon. This strain was selected from mutants obtained by giving *Aspergillus usamii* R-0635 ultra-violet irradiation; it was named *Aspergillus usamii mut. shiro-usamii.* (the prefix "shiro" means white).

2. By further ultra-violet irradiation of this strain an albino type was obtained.

3. Combinations of pairs of all mutants obtained, as well as pairs of mutants with the parent and with other typical Aspergillus strains, were tested for heterocaryosis as shown by the color of conidial heads. Formation of heterocaryotic conidial heads was observed between the tan mutant (*Aspergillus usamii mut. shiro-usamii*) and the brown mutant, between the tan mutant and *Aspergillus usamii* and, and between the brown mutant and *Aspergillus usamii.*

4. Along the interface of tan mutant and brown mutant, black conidial heads which are the same as those of the original strain, *Aspergillus usamii*, develop.

5. Amylolytic and acid producing powers of *Aspergillus usamii mut. shiro-usamii* were found to be as good as those of *Aspergillus usamii*, or better.

6. Favorable results were obtained in tests of an industrial application of *Aspergillus usamii mut. shiro-usamii* for a new type of practical alcoholic fermentation by the Koji method. Therefore this mutant is better for pratical application wheh used with *Aspergillus oryzae* because the mutant has the same features as *Aspergillus usamii* but it is not black.

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STUDIES ON THE ANTI-TRICHOMONAL ACTINOMYCETES AND THEIR CLASSIFICATION

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Even though many distinguished taxonomical studies of the Actinomycetes have been made by Krassilnikov[21] (1941), Waksman[5] (1948), Waksman and Henrici[51] (1953), and others, a standard and decisive classification system has not yet been established. This may be because of the manifoldness and the variability of these microorganisms as well as the lack of adequate schemes for identification. For nearly a decade, therefore, many authors, such as Pridham and Gottlieb[37] (1948), Ludwig and Hutchinson[26] (1949), Hayano[12] (1949), Kuroya et al.[22][23] (1949–50), Okami[33] (1952), Baldacci et al.[1][2] (1953–4), Teillon[44] (1953), Naganishi and Nohmi[29] (1953–5), Hesseltine et al.[14] (1954), Burkholder and Sun[6] (1954), Benedict et al.[3] (1955), Gause[9] (1955), and Couch[7] (1955), have discussed the question and tried to construct keys to make speciation and classification of the Actinomycetes more rational. As for the anti-biotic-producing Actinomycetes, systematic researches were also done by Sakai[40][41] (1954–5), Ogi[31] (1954), Waksman and Gregory[48] (1954), Okami[34] (1955), and others, for anti-Mycobacterial, anti-fungal Actinomycetes, Actinomycin-producing Actinomycetes, and so forth.

Certain strains of *Streptomyces* are known to produce anti-trichomonal anti-biotics, including *S. griseus*[49] (Streptocin), *S. hachijoensis*[16][52], H 3030[52] (Trichomycin), *S. griseolus*[17] (Fermicidin), *S. albireticuli*[10], *S. eurocidicus* (Eurocidin), *S. aureofaciens*[27] (Aureomycin), *S. venezuelae*[27], *S. omiyaensis* (Chloramphenicol), *S. halstedii*[27] (Carbomycin), *S. erythreus*[27] (Illotycin), *S. rimosus*[27] (Terramycin), *S. viridochromogenes*[43] (C-783 substance), *S. erythrochromogenes*[10] (Sarkomycin), *S. thioluteus*[19] (Aureothricin), and others. However, these Actinomycetes have usually been selected by using bacteria or fungi as test organisms and not protozoa themselves. A screening method using protozoa was first reported by Zaher et al.[53] (1953) who chose *Herpetomonas culicidarum*, *Euglena gracilis*, and *Tetrahymena geleii* as test organisms. Saburi[39] (1954) and Okami et al.[35] (1955) followed them, using *Trichomonas vaginalis* and *Toxoplasma*, respectively, as test organisms.

We selected 172 anti-trichomonal Actinomycetes systematically from 1244 Actinomycetes, which include all the typical cultures preserved in

Japan, using *Trichomonas vaginalis* as a test organism. The mycological characters of these anti-trichomonal Actinomycetes and their classification will be described in this paper.

EXPERIMENTAL

1. Screening method.

The "Diphase method", previously reported by one of the authors, was used. Each Actinomycete was grown on a Nutrient agar slant of about 5 cc for 3–5 days, and then 5 cc of Hamada's F broth[18] containing 0.5 cc of a *Trichomonas vaginalis* seed culture was added. After incubating for 20 hours at 37°C, the liquid portion of the diphase medium was examined under a phase microscope, and by counting the number of living trichomonads in the microscopic field we observed the anti-trichomonal activity of each strain of Actinomycetes. If a strain showed anti-trichomonal activity, one portion of its liquid phase was serially diluted with trichomonad-containing Hamada's F broth, and, by incubating as above, we obtained its dilution potency in units. At the same time another portion of the liquid phase was serially diluted with physiological saline and mixed with an equal volume of 1% red blood cell suspension (rabbit blood). We measured the haemolytic power of each strain in this way after incubating for 60 minutes at 37°C. As mentioned previously, we used *Trichomonas vaginalis* T.V. 49–1009 Hamada, as the test organism.

2. Anti-trichomonal Actinomycetes.

Of the 172 anti trichomonal Actinomycetes we selected, 141 cultures were obtained after screening 1,149 strains of Actinomycetes isolated from 350 samples of Japanese soil on modified Krainsky's medium. As shown in Table 1, these soil isolates were found, after mycological studies described in section 3, to belong to 19 species or species groups. It is interesting to note that of these 141 strains 126 strains produced light brown to deep brown soluble pigment on organic medium and only 15 strains did not.

From the results shown in Table 2 it is seen that out of 95 typical Actinomycetes preserved in the Type Culture Collection and Distribution of the Institute of Applied Microbiology, 31 strains were active, and, while all strains belonging to *S. fradiae*, *S. hachijoensis* and *S. roseochromogenus* were active, strains belonging to *S. griseus*, *S. albus* and *S. hygroscopicus* seemed to be either active or inactive. Considering the relationship between morphology of the aerial mycelium and activity, 10 cultures were active among 12 whorl-formers, 15 cultures among 40 spiral formers, 2 cultures among 26 straight aerial mycelium-formers, and 4 cultures among 17 unidentified strains; the proportion of whorl-formers which could produce anti-trichomonal substance seemed to be unusually large. Species or species groups of active strains are shown in Table 1.

Although we obtained active species not previously known to have

Table 1. Anti-trichomonal Actinomycetes.

Species or groups (g.)	New isolates	Stock cultures	Total
S. lavendulae g.	67	5	72
S. reticuli g.	21	7	28
S. diastatochromogenes g.	19		19
S. griseus g.	8	2	10
S. fradiae g.	2	5	7
S. naganishii n. sp. g.	6		6
S. ruber g.	4	1	5
S. hachijoensis g.	2	3	5
S. albus g.	2	1	3
S. purpeochromogenus	2		2
S. hygroscopicus	1	1	2
S. thioluteus		2	2
S. griseoruber n. sp.	1		1
S. farinosus	1		1
S. olbogriseolus	1		1
S. californicus	1		1
S. olivochromogenus	1		1
S. fasciculus	1		1
S. purpeofuscus n. sp.	1		1
S. aureofaciens		1	1
S. aureus		1	1
S. flavochromogenes		1	1
S. cacaoi		1	1
Total	141	31	172

anti-trichomonal activity, such as the *S. lavendulae*, *S. diastatochromogenes*, *S. fradiae*, *S. naganishii* n. sp., and *S. albus* groups, and *S. purpeochromogenus*, we could not get active strains belonging to some known anti-trichomonal species, such as *S. griscolus*, *S. venezuelae*, *S. halstedii*, *S. rimosus*, and *S. viridochromogenes*.

3. Cultural studies of new isolates.

The cultural characters of 141 new isolates of anti-trichomonal Actinomycetes were studied. They were grown in test tubes on a number of different media that are commonly used in the study of Actinomycetes; for the composition of media and cultural conditions, "Cultural Studies of Species of *Actinomyces*" by S. A. Waksman[46] was used as a reference. The type of growth, color of aerial mycelium, soluble pigment formation and other cultural and biochemical characters were noted. Color was chiefly estimated by reference to the "Color Standard and Color Nomen-

Table 2. Anti-trichomonal activity of typical Actinomycetes

Species	Strain	Antibiotic	Activity*
Streptomyces abikoensum	Z–1–6	Abikoviromycin, etc.	0/a
S. albireticuli	3724	Eurocidin, etc.	0/a
S. albogriseolus	NRRL B–1305	Neomycin	1/10
S. albus	ATCC 618		7/1
	IFO 3195		6/1
	ATCC 3381		0/a
S. antibioticus	ATCC 10382		30/1
S. arabicus	6762	Croceomycin	2/1
S. aureofaciens	NRRL 2209	Aureomycin	0/a
S. aureus	ATCC 3309		0/a
S. bobiliae	3310		5/1
S. californicus	ATCC 3312		5/1
S. cinnamonensis	154–T_3	Cinnamonin	1/3
S. coelicolor	ATCC 10147		30/1
	IFO 3114		2/1
	9023		11/1
S. ehimensis	138	Candimycin	0/a
S. flaveolus	ATCC 3319		5/1
	IFO 3241		5/1
	W– 36	Flaveolin	4/1
	H– 3185	〃	2/1
S. flavochromogenes	H– 3206	Vulgarin	0/a
S. flavovirens	ATCC 3320		1/1
	IFO 3197		19/1
S. flavus	134	Actinomycin J	6/1
S. fradiae	3535	Neomycin, etc.	0/a
	NRRL B–1195	〃	0/a
	IFO 3123	〃	0/a
	260	〃	0/a
	117	〃	0/a
S. griseocarneus	NRRL B–1068	Hydroxystreptomycin	0/a
S. griseolus	3325		16/1
	NRRL B–1062		6/1
S. griseoluteus	P– 37	Griseolutein	5/1
S. griseus	3463	Streptomycin	0/a
	B_3	〃	1/5
	G– 24	〃	10/1
	41	〃	4/1
	H– 12	Grisein	5/1
	Matsudaira		0/a
S. griseus var. rhodochrous	SN_1 J		9/1
	SN– 14		5/1
S. hachijoensis	H– 2609	Trichomycin	0/a
	H– 2552	〃	0/a
	H– 2635	〃	0/a
S. halstedii	IFO 3199		20/1
S. hygroscopicus	IFO 3192		20/1
	6A– 704	Angstmycin	0/a
	1545	Hygroscopin	15/1
S. kitasatoensis	S. 299– 5	Leucomycin	0/a
S. lavendulae	ATCC 8664	Streptothricin	0/a
S. lavendulae var. japonicus	E– 2	〃	0/a
S. lipmanii	3331		1/10
S. microflavus var. antibioticus	13–A	Streptothricin	5/1
S. nitrosporeus	0–20	Nitrosporin	6/1
S. olivaceus	NRRL B–1125		1/10
	IFO 3200		3/1
S. olivochromogenus	3292	Virosin	6/1
S. parvus	NRRL B–1255		3/1

Species	Strain	Antibiotic	Activity*
S. phaeochromogenus	ATCC 3338		2/1
S. phaeofaciens	108–A1	Phaeofacin	9/1
S. purpurascens	NRRL B–1454		6/1
S. rimosus	NRRL 2234	Terramycin	5/1
S. roseochromogenus	O–36–748	Roseomycin	0/a
	O–20–316	〃	0/a
	H–277	Roseothricin	0/a
S. ruber	IFO 3110		3/1
S. rubescens	Z–5–2	Abikoviromycin	7/1
S. rubrireticuli	3631		10/1
S. rutgersensis	3350		7/1
S. scabies	IFO 3111		1/1
S. sindenensis	1071	Amicetin	2/1
S. tanashiensis	144	Luteomycin	2/1
	H– 2053	〃	1/5
	289–A1	289 substance	5/1
S. thioluteus	26A	Aureothricin	0/a
	280–3	〃	0/a
S. venezuelae	75	Chloramphenicol	1/3
	213	〃	4/1
S. vinaceus	NRRL B–1381	Vinactin	2/1
S. virginiae	NA 255–M 25	Actithiazic acid	2/1
S. viridochromogenes	IFO 3113		10/1
Z–3–5	Z–3–5	Exfoliatin	8/1
H–662	H– 662	Xanthomycin	4/1
H–1159	H– 1159	〃	1/1
S. reticuli group	H– 365	Reticulin, etc.	0/a
G–286	G– 286	Anti–607 substance	2/1
G–394	G– 394	〃	5/1
G–167 (resembling to *S. cacaoi*)	G– 167	〃	0/a
H–3030 (belonging to *S.reticuli* g.)	H– 3030	Trichomycin	0/a
H–2491 (〃 〃 *S. ruber* g.)	H– 2491	Anti-fungal substance	0/a
Nocardia asteroides	3308		4/1
N. gardneri	9604	Proactinomycin	3/1
Micromonospora sp.	3453		3/1
Thermoactinomyces vulgaris	3397		11/1
Control			5/1–30/1

* The numerator shows the number of moving trichomonads and the denominator shows the number of microscopic fields. Denominator "a" means that 30 or more microscopic fields of the specimen were examined. Thus, 0/a shows complete anti-trichomonal activity of an Actinomycete.

clature" of Ridgway[38] (1912). At least two series of tests were made, each employing duplicate tubes or plates of medium. Media were inoculated by streaking. All cultures were kept at 25°C for 15 days incubation period, unless otherwise noted. For species and species group identification much information is given in "Actinomycetes and Their Antibiotics" by Waksman and Lechevalier[51] (1953), but we endeavored so often as possible to refer to original descriptions such as those of Krainsky[23], Waksman[46,47], Lindenbein[25] and others, and we tried to include new species or strains reported recently.

I) Strain H-5080

(1) Synthetic solution

Whitish growth is scattered all through the medium. Aerial mycelium is whitish on the surface. Trace of purplish soluble pigment is produced. Reduction of nitrate is negative, and there is almost no change in pH.

(2) Synthetic agar

Growth is at first thin, colorless, later becoming purple to dark purple, but this property may be lost after repeated transfer, in which case it remains white to light purple. Aerial mycelium is powdery, at first white, later changing to smoke gray. Faint purple soluble pigment is produced.

(3) Krainsky's agar

Growth is very good, with at first reddish brown, later blackish, reverse. Abundant, velvety, white, later, light grayish-olive aerial mycelium is produced. There is a trace of soluble pigment.

(4) Ca-malate Glycerine agar

Brownish growth is covered with white, smoke gray to light grayish-olive aerial mycelium. Reverse is deep purplish-brown. Light brownish-purple soluble pigment is produced.

(5) Nutrient agar

Growth is at first colorless, but soon shows soluble pigment production; culture is wrinkled with a rough surface. Aerial mycelium is missing or scanty, white. Soluble pigment changes from reddish brown, army brown to dark vinaceous-brown.

(6) Egg medium (37°C)

Growth is finely wrinkled, at first light brownish-purple, later brownish-purple. Aerial mycelium is faint, whitish. Faint purple soluble pigment is produced along the margin. No liquefaction is observed.

(7) Blood agar (37°C)

Growth is at first glossy, deep gray, but later changes to grayish or colorless. There is no serial mycelium. At first the medium becomes light blackish, but later this color is lost. Haemolytic action is weak.

(8) Potato plugs

Growth is vigorous, elevated, finely wrinkled, at first purplish-brown or yellowish-brown, later becoming black. Abundant, powdery, grayish, aerial mycelium and purplish soluble pigment are produced.

(9) Starch agar

Growth is yellowish-brown, dark olive buff to buckthorn brown and sometimes has hygroscopic, black patches. Reverse is fuscus. Aerial mycelium is velvety, at first white, later olive-gray. Usually no soluble pigment is produced, but sometimes faint pinkish purple is seen. Zone of enzyme action is 20–22 mm wide (15 days).

(10) Gelatine

Growth is dark brownish-gray with brownish gray to blackish reverse. Coarse, powdery, grayish-white aerial mycelium is produced. Soluble pigments are yellowish-brown to brown and a more diffusible yellowish-green.

Liquefaction is strong.

(11) Milk (37°C)

Growth is at first dull reddish brown, later purplish brown, but at 25°C color of growth is olive gray, sometimes very slowly changing to reddish-brown. No aerial mycelium is produced. Soluble pigments are faint purplish and more diffusible faint yellowish colors at 37°C, while at 25°C they are faint yellowish green to faint yellowish gray. Coagulation begins in 3 days and peptonization proceeds very slowly. pH changes to somewhat alkaline reaction.

(12) Paper Strip Synthetic Solution — no growth takes place.

(13) Tyrosinase reaction is negative.

(14) Microscopic observation:

Aerial hyphae are long and straight, and on Synthetic agar and on Starch agar they show a tuft-forming tendency in the margin. Spirals are not produced. Conidia are cylindrical, 1.1–2.2 $\mu \times 0.7$–1.1 μ.

(15) Dilution potency against *T. vaginalis* is more than 128 units, and haemolytic power is 0.

Comparing above mycological characters with those of known species, H-5080 somewhat resemble *S. vinaceus*, *S. cylindrosporus*, *S. purpeochromogenus*, but differs from them in the following points. *S. vinaceus* does not produce soluble pigment on Nutrient agar, Gelatine, and Potato plugs, and color of growth on Starch agar is off-white. Description of *S. cylindrosporus* seems to be incomplete and differs with H-5080 chiefly in biochemical characters, such as diastatic action, nitrate reduction, and sucrose utlization. Aerial mycelium of *S. purpeochromogenus* has imperfect spirals on Starch agar and is not produced on Synthetic agar, and its color of growth on Potato plugs is orange to orange red. Thus H 5080 does not conform with any known species and seems to constitute a new species; we have named it *S. purpeofuscus* n. sp., which has following characters: Straight, white to gray aerial mycelium with tuft forming tendency in the margin; vegetative mycelium on synthetic media white, purple to brown, with various shades of gray, yellow, and red; soluble pigment formed on organic media is light purple, reddish brown, purplish brown, someties yellowish-brown.

II) Strain H-3882

(1) Synthetic agar

Colorless, thin growth penetrates deep into the medium. Aerial mycelium is powdery, at first white, later smoke-gray. There is no soluble pigment.

(2) Krainsky's agar

Rapid, vigorous growth is quickly covered with powdery, whitish-gray to mineral-gray aerial mycelium, and has some droplets. Reverse is yellowish-white to yellowish. Pale yellowish-green soluble pigment is slowly produced.

(3) Nutrient agar

Growth is vigorous, elevated, and rapidly covered with abundant white to grey aerial mycelium. Reverse is yellowish-brown. Soluble pigment is not produced.

(4) Blood agar (37°C)

Growth is grayish-olive. There is usually no aerial mycelium, but sometimes faint, pale yellowish-gray aerial mycelium is produced. Soluble pigment is black. Haemolytic action is strong.

(5) Potato plugs

Growth is vigorous, elevated, wrinkled, light greenish-yellow, and quickly covered with powdery, white to gray aerial mycelium. Sometimes droplets are produced. Plugs change to somewhat darker color.

(6) Starch agar

Growth is quickly covered with powdery, white, later gray, aerial mycelium. Droplets are produced. Reverse is light yellowish-brown. Solube pigment is absent or faint yellowish-green. Zone of enzyme action is 26–28 mm wide (15 days).

(7) Gelatine

Reverse is yellowish to greenish-yellow. Aerial mycelium is whitish-gray and has droplets. There is no soluble pigment or only a trace.

(8) Milk (37°C)

Growth is creamy to light yellowish on the surface, and no aerial mycelium is produced. (White, powdery aerial mycelium is produced at 25°C.) Soluble pigment is missing or sometimes light yellowish-brown. Coagulation begins in 5 days and peptonization proceeds. pH reaction changes to somewhat alkaline.

(9) Microscopic observation

Aerial hyphae are straight, and on Synthetic agar, Krainsky's agar and Starch agar there is a tuft-forming tendency in the margin. Spiral hyphae are not observed. Conidia are cylindrical to short rod, 0.9–1.2×0.5–0.9 μ.

(10) Dilution potency against *T. vaginalis* is less than 2 units, and haemolytic power is 0.

Comparing H-3882 with known species, there is some resemblance to *S. fasciculus*, *S. farinosus*, *S. olivaceus*, *S. canescus* and *S. nitrosporeus*.[32] This strains seems to resemble most *S. fasciculus* (Krasslinikov) Waksman and Henrici, though we find some difference in cultural characters on Nutrient agar and Potato plugs.

III) Strains H-5109, H-3849, H-4038, H-3885, H-3902, H-3831, H-3886, H-4071 (8 strains—Type culture H-4038)

(1) Synthetic agar

Growth is at first colorless, thin, later with light yellowish to chamois reverse. Aerial mycelium is powdery, light greenish-yellow. Usually no soluble pigment is produced, but sometimes faint greenish yellow soluble pigment is found in very old cultures.

(2) Nutrient agar

Growth is vigorous, at first colorless, later becoming creamy to yellowish-cream. Most strains produce lateral furrows. Some strains produce white to faint greenish-yellow aerial mycelium abundantly, but others have mycelium only in patches or not at all. Usually no soluble pigment is produced, but sometimes faint brown pigment is produced in old cultures.

(3) Starch agar

Growth is colorless to creamy with abundant, white to faint greenish-yellow, aerial mycelium. There is no soluble pigment or only a trace.

(4) Microscopic observation

Aerial hyphae are straight, long or short, with a tuft-forming tendency in the margin. Sometimes aerial hyphae branch irregularly but they are rarely wavy. Spirals are not produced.

(5) Dilution potency against *T. vaginalis* is less than 2 units, and haemolytic power is 0 in all strains.

The above 8 strains undoubtedly belong to Waksman's *S. griseus* group. The cultural characters of H-4038 on various other media have been examined, and we find it belongs to *S. griseus* (Waksman and Curtis) Waksman and Henrici.

IV) Strains H-4773, H-4813, H-4280, H-4911, H-5024, H-4440, H-4827, H-4520, H-5044, H-4726, H-5058, H-5077, H-4512, H-4922, H-4490, H-4576, H-4713, H-4556, H-4684, H-4950, H-4464 (21 strains—Type culture H-5044)

(1) Synthetic agar

Growth is spreading and penetrates deep into the medium; at first colorless, later marguerite yellow to primrose-yellow, sometimes pale dull green-yellow. Aerial mycelium is slowly produced, cottony, white, later light tan to light pinkish in very old cultures. However, a few strains such as H-4490 produce faintly yellowish, not cottony, aerial mycelium. Amount of aerial mycelium produced is changeable by minor cultural conditions, and some strains, such as H-4726, may produce none. Soluble pigment is not produced.

(2) Nutrient agar

Growth is very vigorous, elevated; at first glossy, creamy, later lichenoid with increasing greenish color in most strains, and brownish color in a few strains. Aerial mycelium is slowly produced, powdery, white with a shade of yellowish-green. Sometimes a few strains like H-4684 and H-4950 produced powdery aerial mycelium quickly, but this character is not stable. Soluble pigment is brown.

(3) Starch agar

Growth is spreading, colorless, later creamy, with pale brownish portion in the center of colony where aerial mycelium is not produced. Aerial mycelium is cottony, white, produced from the margin. No soluble pigment or only a trace is found.

(4) Microscopic observation

Aerial hyphae are straight, and produce a few primary and secondary whorls, or some imperfect whorls having only a few irregular branches. When aerial mycelium production is limited by minor conditions only straight hyphae and no whorls are observed. In some media, or in some strains having faintly yellowish, not cottony, aerial mycelium, aerial hyphae show complicated figures and no whorls are produced. Whorls are produced on Synthetic agar better than on Starch agar. No spirals are observed, and conidophores are straight.

(5) Dilution potency against *T. vaginalis* is 16–128 units (average 64), and haemolytic power is 8–128 units (average 64). This group shows the highest average dilution potency and haemolytic power.

The above 21 strains and the following 2 strains are all whorl formers and not only are they conspicuous morphologically, but biochemically they also seem to have some similarity between them, such as a narrow range of carbon utilizability, and so forth. As whorl-formers, aside from *S. circulatus* and *S. netropsis*[8] which have spiral sporophores, there are *S. reticuli*, *S. rubrireticuli*, *S. verticillatus*, *S. griseocarneus*[15], *S. hachijoensis*[16)52)], *S. viridoflavus*, *S. kitasatoensis*[11)], *S. mediocidicus*,[36)] *S. eurocidecus*[36)], *S. cinnamoneus*[8)], *S. ehimensis*, *S. mashuensis*[42)], *S. albireticuli*[30)] and *S. abikoensum*.* They include various species—some produce brown soluble pigment, others do not; the vegetative mycelium varies from colorless, yellowish with various shades of brown or green, or even to red, but the color of cottony aerial mycelium of all whorl-forming species is white, light tan, pale pinkish buff to pale cinnamon pink. The authors propose to call chromogenic whorl formers, such as *S. reticuli*, *S. rubrireticuli*, *S. verticillatus*, *S. griseocarneus*, *S. viridoflavus*, *S. kitasatoensis*, *S. mediocidicus*, *S. eurocidicus*, *S. ehimensis*, *S. albireticuli* and *S. abikoensum*, *S. reticuli* group, and to all non chromogenic whorl-formers, such as *S. hachijoensis*, *S. cinnamoneus*, and *S. mashuensis*, *S. hachijoensis* group. Species identification in both groups seems to require further consideration, so we tentatively place the above 21 strains with the *S. reticuli* group.

V) Strains H 4278, H-4592

(1) Synthetic agar

Growth is spreading and penetrates deep into the medium; colorless in H-4278, light dull yellow in H 4592. These produce white, cottony aerial mycelium, but in H-4592 production is only fair. Soluble pigment is not formed.

* The aerial mycelium of *S. abikoensum* Umezawa, Tazaki, and Fukuyama[45)] is reported to be "microscopically short, unbranched, bearing a straight conidia" and no description of whorl formation is given. We inoculated *S. abikoensum* Z-1-6 (Type culture) on Synthetic agar containing commercial sugar as a carbon source, and observed abundant typical primary whorl formation of aerial hyphae.

(2) Nutrient agar

Growth is vigorous. H 4278 has whitish cream growth at first, soon covered with white aerial mycelium having yellowish brown reverse; a trace of soluble pigment or none is produced. H-4592 has dull yellow, light olive to greenish cream growth, scanty pale olive-white aerial mycelium, and faint brown soluble pigment or none.

(3) Starch agar

Growth is at first colorless, later becoming yellowish. Aerial mycelium is cottony, white in H-4278, and light yellowish white in H 4592. Soluble pigment is not formed.

(4) Microscopic observation

Aerial hyphae are straight and form straight sporophored primary and secondary whorls. H 4592 produces more whorls than H 4278. No spirals are observed.

(5) Dilution potency againt *T. vaginalis* is 8 units (H 4278) and 64 units (H 4592), and haemolytic power is 64 units in both strains.

The above 2 strains do not produce brown soluble pigment on organic medium and so belong to *S. hachijoensis* group as discussed in Sect IV.

IV) Strain H-3845

(1) Synthetic agar

Growth is colorless and thin, with powderly, light drab to smoke gray aerial mycelium. Soluble pigment is not formed.

(2) Krainsky's agar

Reverse is yellowish-brown to brownish and powdery, whitish gray aerial mycelium is produced. Soluble pigment is at first yellow, later brownish.

(3) Nutrient agar

Flat and laterally furrowed growth is quickly covered with abundant, white aerial mycelium. Reverse is cinnamon-rufous. Light brown soluble pigment is formed.

(4) Potato plugs

At first growth is elevated, wrinkled, and yellowish, but it is quickly covered with grayish white aerial mycelium. Droplets are seen. Deep purple to black soluble pigment is produced.

(5) Starch agar

Growth is at first antique-brown to natal-brown, but soon covered with aerial mycelium. Reverse is deep brown. Aerial mycelium is at first white later changes to drab gray to deep olive-gray. Solube pigment is at first yellowish brown, later deep brown. Zone of enzyme action is 18–20 mm wide (15 days).

(6) Gelatine

Reverse is yellowish-brown to brown. White to faintly yellowish-white aerial mycelium is produced. Brown, and more diffusible dull yellow, soluble pigments are formed. Liquefaction is strong.

(7) Milk (37°C)

Surface pellicle is at first light creamy to light brown, but later reverse becomes brownish to black. Scanty, grayish white, aerial mycelium is produced. Soluble pigment is deep brown to black. Coagulation begins in 3–5 days and rapid peptonization occurs.

(8) Microscopic observation

Aerial hyphae are filamentous and form closed spirals.

(9) Dilution potency against *T. vaginalis* is less than 2 units, and haemolytic power is 0.

Comparing the above cultural findings with those of known species, H-3845 somewhat resembles *S. olivochromogenus*, *S. resistomycificus*, *S. phaeochromogenus*, *S. aureus* and *S. phaeofaciens*[28], and especially because of similarity of the color and morphology of the aerial mycelium, the color of the vegetative mycelium, and soluble pigment production, it seems to resemble *S. olivochromogenus* (Bergey et al.[5]) Waksman and Henrici, though we find differences in some cultural characters.

VII) Strains H-5059, H-4742 (2 strains—Type culture H-5059)

(1) Synthetic agar

Growth is at first colorless to faintly creamy, but with production of soluble pigment it changes to light purplish color. Aerial mycelium is usually scanty, white, but in very old cultures it is powdery, deep mouse-gray to drab. Soluble pigment is usually faint purple, but in very old cultures it changes to deep bluish purple.

(2) Nutrient agar

Growth is at first glossy, colorless to grayish-white smear, but latter shows increasing brownish color with light brown soluble pigment. Aerial mycelium is not produced.

(3) Starch agar

Growth is vigorous, spreading, at first colorless, light navy blue, changing to purplish-black to bluish black. Aerial mycelium is abundantly produced, powderly, mouse gray to drab. Faint dark-bluish soluble pigment is produced.

(4) Microscopic observation

Aerial hyphae are long, filamentous, and produce many closed spirals.

(5) Dilution potency against *T. vaginalis* is 2 units or less than 2 units, and haemolytic power is 0.

The cultural characters of H-5059 on other media have been observed and we find that it belongs to *S. purpeochromogenus*, though its cultural characters on Potato plugs are somewhat different. As shown in Table 3, the carbon utilization of H-4742 is also the same as that of H 5059; both strains seem to belong to *S. purpeochromogenus* (Waksman and Curtis) Waksman and Henrici.

VIII) (a) Strains H-4389, H-4788, H-4388, H-4314, H-4884, H-4336, H-5072, H-4803, H-4608, H-4791, H-4614, H-4651, H-4891, H-4422, H-4619, H-4732, H-5115, H-4821, H-5114, H-5102, H-4620, H-4782

(22 strains—Type culture H-4422)

(b) Strains H-4989, H-5066, H-4990, H-4660, H-4380, H-4545, H-4491, H-4394, H-4374, H-5065, H-4579, H-4738, H-4689, H-4524, H-4590, H-4632, H-4829, H-4941, H-4815, H-4683, H-5061, H-4552, H-4382, H-5002, H-4403, H-4790, H-4375, H-4957, H-4733, H-4583, H-4852, H-4711

(32 strains—Type culture H-5002)

(c) Strains H-4392, H-4519, H-4371, H-4644, H-4596, H-4807, H-5065, H-4642, H-4662, H-4637, H-4517 (11 strains—Type culture H-4519)

(d) Strains H-4778, H-5023 (2 strains—Type culture H-4778)

The above strains listed under a, b, c, and d have many common characters and seem to be related to one another, so we will describe them together.

(1) Synthetic agar (Commercial sugar was used as carbon source.)

Growth: As a and b do not utilize saccharose their growth is thin, and colorless to faintly creamy, whereas c and d utilize saccharose and their growth is good and thick. When newly isolated, c is colored by soluble pigment production, but this character is lost by repeated transfer and only H-4519 and H-4392 show buffy brown color. Reverse of H-4644, H-4596, H-4807, H-4662, H-5065, H-4517 is light cream, reverse of H-4637, H-4642, H-4371 is yellow, and reverse of H-4519, H-4392 is light purplish-brown. Color of growth of d changes to light yellowish green by soluble pigment production, and the reverse is yellow to light yellowish brown.

Aerial mycelium: Color of aerial mycelium of a, b, and d is usually pale vinaceous-pink, but H-5115, H-4821 and H-4114 have light vinaceous-fawn color, and H-5102 has light congo-pink color. Aerial mycelium of a and d is powdery, and that of b is velvety to cottony. After repeated transfer, aerial mycelium production of b becomes limited and its color of d becomes white. When newly isolated, aerial mycelium of c was light pinkish with light navy blue portion, with soluble pigment production, but becomes scanty to fair, velvety to cottony, white.

Soluble pigment: No soluble pigment is formed in a or b. c has light wine-colored soluble pigment when newly isolated, but later changes to colorless, faint blue, faint purple to faint brown. d produces light yellowish green soluble pigment.

(2) Nutrient agar

Growth: a is vigorous, at first glossy, grayish cream, later changing to deep gray to brownish color. b is almost the same as a, but somewhat restricted, changing to deep olive-buff. In exceptional cases, H-4711, H-4852, H-4394, and H-4815 are finely wrinkled and changing to dark greenish-yellow. Strains belonging to c and d are at first creamy, later discolored to dull tint in c, but with increased brownish tone in d.

Aerial mycelium: Usually none, but sometimes scanty, white, though

this character is quite changeable.

Soluble pigment: a, b, c and d produce light brown to deep brown, but only H-4517 produces faint brown soluble pigment.

(3) Starch agar

Growth: Vigorous; colorless to creamy in a, b and d; c frequently produces light bluish sectors with brownish blue reverse.

Aerial mycelium: White, cottony, appearing from the margin, but later becoming light pinkish. H-5102 produces aerial mycelium very quickly.

Soluble pigment: None in a, b, and d; c sometimes produces faint bluish, soluble pigment.

(4) Microscopic observation

On Synthetic agar and on Starch agar, aerial hyphae produce abundant closed spirals in a and a few in b. c and d produce very few closed spirals and rarely open or compact spirals.

(5) Dilution potency against *T. vaginalis* is less than 2 units, except for a few strains in a and d, but 4 to 8 units in most strains of b and c. Haemolytic power is 0 to 1 unit except for a few strains in a and d, but 8 to 16 units in most strains of b and c.

According to the above cultural characters and carbon utilization as shown in Table 3, strains beloging to a, b, c, and d seem to constitute a group with the characteristics of formation of predominantly closed spirals on a white to light pinkish aerial mycelium, and production of brown soluble pigment on protein media; they thus to conform with Waksman's *S. lavendulae* group. The cultural characters of H-4422. H-5002, H-4519, and H-4778 on other media have been observed and these confirm the identity.

IX) Strains H-3904, H-3798 (2 strains—Type culture H-3904)

(1) Synthetic agar

Growth is thin, colorless. Aerial mycelium is powdery, seashell pink in H-3904 and light ochraceous-buff in H-3798. Soluble pigment is not produced.

(2) Nutrient agar

Growth is vigorous, whitish-cream to grayish-cream. Aerial mycelium is absent or scanty, white. Soluble pigment is not produced.

(3) Starch agar

Growth is at first colorless to creamy, later becoming orange. H-3904 produces powdery, pale grayish-vinaceous aerial mycelium and H 3798 produces powdery white aerial mycelium.

Soluble pigment is faint yellow to faint orange.

(4) Microscopic observation

Aerial hyphae of H-3904 produce abundant closed spirals and very few open spirals of 3 to 5 turns. Aerial hyphae of H 3798 are wavy, and have tuft-forming tendency.

(5) Dilution potency against *T. vaginalis* is 8 units in H-3904 and less than 2 units in H 3798. Haemolytic power in both is 0.

Further studies on various other media were made and they show that these two strains belong to Waksman's *S. fradiae* group.

X) Strains H-4725, H-4624 (2 strains—Type Culture H-4725)

(1) Synthetic agar

Growth is spreading, thick, and at first colorless, jasper red to flame-scarlet, later with coral red reverse. Aerial mycelium is absent, or scanty, white. Soluble pigment is not formed.

(2) Nutrient agar

Growth is at first grayish-white, later changing to dark cream to brownish color following brown soluble pigment production. Aerial mycelium is absent or scanty, white.

(3) Starch agar

Growth is spreading, colorless, rose dorée, later congo-pink or somewhat purplish color in reverse. Aerial mycelium is cottony, white. Soluble pigment is light ochraceous-salmon, often only a trace. Colonies on plates have 12–22 mm wide zone of enzyme action (15 days).

(4) Microscopic observation

On Starch agar and on Glycerine agar, aerial hyphae are filamentous and produce open spirals.

(5) Dilution potency against *T. vaginalis* is 2 to 16 units, and haemolytic power is 0 to 2 units.

The cultural characters on other media also have been observed and we find that these strains resemble *S. ruber* and *S. aurantiacus*, and belong to Waksman's *S. ruber* group.

XI) Strain H-4156

(1) Synthetic solution

Growth is slow, white, and producing white aerial mycelium on the surface. Soluble pigment is not formed. There is no nitrate reduction.

(2) Synthetic agar

Growth is slow, colorless, thin, with powdery, white aerial mycelium. Soluble pigment is not formed.

(3) Krainsky's agar

Growth is glossy, creamy-white, and aerial mycelium is produced. Soluble pigment is at first light yellowish-green, later greenish-yellow.

(4) Nutrient agar

Growth is creamy-white and there is a trace of aerial mycelium or none. Soluble pigmen is not present.

(5) Blood agar (37°C)

Growth is at first pale yellow to yellowish-gray, later changing to grayish-olive. Aerial mycelium is produced only at top of slant, white. Medium slowly turns dark. Haemolytic action is positive.

(6) Potato plugs

Growth is elevated, wrinkled, and at first faint yellowish-green to yel-

lowish, but soon covered with grayish or pale yellowish-white aerial mycelium. Soluble pigment is faint purple or absent.

(7) Starch agar

Reverse is light cream. White aerial mycelium is produced. Soluble pigment is not present. Colonies on plate have 8–12 mm wide zone of enzyme action (15 days).

(8) Gelatine

Growth is mainly on the bottom, and scanty, colorless to faint cream on the surface. Aerial myceliun and soluble pigment are not produced. Liquefaction is strong.

(9) Milk (37°C)

Growth is light yellowish. Aerial mycelium and soluble pigment are not produced. Coagulation begins in 7 days and peptonization proceeds very slowly.

(10) Microscopic observation

On Synthetic agar aerial hyphae are straight, and produce many short, wavy or compact spiral-like branches.

(11) Dilution potency against *T. vaginalis* is less than 2 units, and hae molytic power is 0.

Comparing the above cultural characters with those of known species, H-4156 resembles *S. farinosus*, *S. candidus* and *S. gougeroti* in some ways, and among these three it seems most like *S. farinosus* (Krassilnikov) Waksman and Henrici.

XII) Strains H-4871, H-4870, H-4868, H-4447, H-4448, H-4869 (6 strains—Type culture H-4871)

XIII) Strains H-4185, H-4161, H-4139, H-4090, H-4296, H-4655, H-4630, H-5018, H-4678, H-3889, H-4528, H-4283, H-5096, H-4024, H-3962, H-4413, H-4172, H-4168, H-4640 (19 strains—Type culture H-4185)

The above strains belonging to XII) and XIII) have many common characters, and seem to related to each other, so we will describe them together.

(1) Synthetic agar (Comercial sugar was used as carbon source.)

Growth: As strains belonging to XIII) utilize saccharose, their growth is thick, colorless, white, yellowish white to yellow, with yellowish white to light brown reverse. Those included in XII) do not utilize saccharose, and their growth is thin, colorless.

Aerial mycelium: Aerial mycelium of XII) is powdery, at first white later drab-gray to tilleul-buff. XIII) usually forms none or scanty, white aerial mycelium, but sometimes produces grayish-white to light drab aerial mycelium in very old cultures.

Soluble pigment: None. Cream-buff soluble pigment is produced rarely.

(2) Nutrient agar

Growth: Vigorous, at first colorless to creamy, later increasing brown-

ish to grayish olive color. Sometimes it is radially wrinkled or laterally furrowed. Individual colony has a tendency to form center depression.

Aerial mycelium: None, or sometimes scanty, white. This character is changeable and sometimes good, white aerial mycelium is produced.

Soluble pigment: Brown.

(3) Starch agar

Growth: At first colorless, later creamy, with faint yellow to light yellowish-brown reverse. But H-4871 and H-4870 produce purplish-vinaceous portion in reverse.

Aerial mycelium: Cottony to powdery, at first white, later changing to light drab to drab, produced from the margin. But H-4655 and H-4630 have powdery, mineral-gray aerial mycelium.

Soluble pigment: None, but H-4871 and H-4870 produce faintly pinkish soluble pigment when their growth has purplish-vinaceous portion in reverse.

(4) Microscopic observation

Aerial hyphae of XII) produce abundant compact spirals and a few open spirals on various synthetic media. Aerial hyphae of XIII) produce abundant open spirals on Starch agar, etc. But aerial hyphae of a few strains, such as H-4413, are irregularly branching and only a few spirals are observed.

(5) Dilution potency against *T. vaginalis* is 8–128 units in XII), and less than 2 to 4 units (sometimes 64) in XIII). Haemolytic power is 0–128 units in XII), and 0 in XIII).

The above 25 strains grouped under XII) and XIII) all have white, light drab to drab gray, aerial mycelia and produce brown soluble pigment on organic media; morphologically they seem to belong to one group in which the typical open spiral-forming strain H-4185, and the typical compact spiral-forming strain, H 4871, are two extremes. Further studies of the typical strains H-4871, H-4869, H-4185, H-4630, H-4413 and H-4640 on other media, and carbon utilization tests, show that H-4871 belongs to *S. naganishii* n. sp., and strains of XII) also are included in the *S. naganishii* n. sp. group. H-4185, H 4630, H-4413, and H-4640, however, resemble such species as *S. diastatochromogenes*, *S. canus*[13], the *S. fungicidicus* group G[36], and *S. diastaticus*, and, as the description of *S. diastatochromogenes* is oldest, we assign the strains of XIII) to that group.

Cultural characters of H-4871 are as follows.

(1) Synthetic solution

Growth is white and chiefly on the surface. Aerial mycelium is white and soluble pigment is missing. Ability to reduce nitrates and change in pH are very small.

(2) Synthetic agar

Growth is colorless and thin, producing powdery, at first white, later tilleul-buff aerial mycelium. Soluble pigment is not found.

(3) Krainsky's agar

Growth is colorless, thick, with plumose margin, penetrating deep into

the medium, and producing white, aerial mycelium at bottom of slant. Soluble pigment is at first faint pinkish, but later disappears.

(4) Ca-malate agar

Growth is at first pinkish-white to pinkish-gray, later becoming whitish light brown in reverse. Aerial mycelium is whitish. Soluble pigment is light pink but soon disappears.

(5) Nutrient agar

Growth is at first colorless to dark cream, later becoming yellowish-brown to brown. Aerial mycelium is not formed. Soluble pigment is light brown.

(6) Egg medium (37°C)

Growth is vigorous, light creamy with lateral folding, later turning to somewhat dark color. White aerial mycelium and purple zone around growth are formed.

(7) Blood agar (37°C)

Growth is restricted, at first grayish-olive, later becoming pale olive. Aerial mycelium is not produced. Dark soluble pigment is produced. Haemolytic action is positive.

(8) Potato plugs

Growth is vigorous, at first yellowish-gray, but soon covered with white to grayish-white aerial mycelium. Deep purple to black soluble pigment is produced.

(9) Starch agar

Reverse is colorless to creamy with reddish purple portion. Aerial mycelium is abundant, white, smoke gray to light drab. Soluble pigment is absent or faint pink. Zone of enzyme action on agar plate is 13–16 mm wide (15 days).

(10) Gelatine

Growth is dark brown with some tint of olive. Aerial mycelium is not produced. Soluble pigments are a deep brown and a more diffusible yellowish-green. Liquefaction is moderate.

(11) Milk (37°C)

Growth is vigorous, yellowish brown, with white aerial mycelium along the glass. Soluble pigment is light brown, but at 25°C sometimes reddish-brown pigment is produced. Coagulation begins in 3 days and peptonization proceeds.

(12) No growth is observed on Paper Strip Synthetic Solution.

(13) Tyrosinase reaction is negative.

(14) Dilution potency against *T. vaginalis* is 8 units, and haemolytic power is 0.

(15) As seen in Fig. 6, aerial hyphae are straight and have many compact spirals, with a few open spirals exceptionally. On Synthetic agar and on Starch agar, they are long and straight, and conidiophores are mainly produced singly as short, lateral branches of main hyphae, showing globose, or sub globose compact spirals, especially in the margin. Compact spirals

are 2.5–4.0 μ in diameter and their conidia are oval to short rod shapes, 0.8–1.4×0.5–0.7 μ. No sporangium or zoospore is observed.

The above mycological characters do not conform with those of any other known species, so we classify H-4871 as a new species, *Streptomyces naganishii* n. sp., named after Prof. Naganishi, who described *Streptomyces* sp. No. 240 Naganishi and Nohmi[29], a very similar strain except for the color of the aerial mycelium. In the morphology and color of aerial mycelium, *S. naganishii* n. sp. resembles *Streptomyces* sp. Leben, Stessel, and Keitt[24], but differs in the points of brown (and sometimes faint pink) soluble pigment production, formation of reddish-purple portions on some synthetic media, and cultural characters on Malate-glycerine agar and on Nutrient agar. *S. naganishii* n. sp. has following characteristics: Aerial mycelium is white to light brownish-gray and has abundant compact spirals. Produces light brown to brown soluble pigment on organic media and sometimes faint pinkish soluble pigment on synthetic media. Vegetative mycelium is colorless, whitish, faintly yellowish or sometimes with reddish-purple portion on synthetic media.

XIV) Strains H-4650

(1) Synthetic agar

Growth is reddish orange when newly isolated, but changes to colorless or whitish after repeated transfer. Aerial mycelium is powdery, drab-gray. There is no soluble pigment.

(2) Krainsky's agar

Growth is thick, flame-scarlet and usually with grayish or faintly reddish aerial mycelium, but sometimes no aerial mycelium is produced. Soluble pigment is almost absent or faint reddish-orange.

(3) Ca-malate agar

Growth is at first glossy, pinkish-gray, later becoming dull-purplish color in reverse. Aerial mycelium is powdery, olive gray. Soluble pigment is missing or faint yellowish-brown.

(4) Nutrient agar

Growth is glossy, at first light olive gray, later becoming brown. Aerial mycelium is absent or scanty, white. Brown soluble pigment is produced.

(5) Glucose broth

Growth is chiefly on the surface, grayish yellow-brown to brown. Aerial mycelium is white, and soluble brown pigment is formed.

(6) Egg medium (37°C)

Growth is radially wrinkled, grayish olive, and no aerial mycelium is formed. Deep purple soluble pigment is produced.

(7) Blood agar (37°C)

Growth is flat, laterally furrowed, olive gray, and aerial mycelium is not produced. Soluble pigment is black. Haemolytic action is positive.

(8) Potato plugs

Growth is elevated, wrinkled, and at first olive-gray to dark yellowish-

brown, later becoming dark reddish color or black. Aerial mycelium is absent or scanty, white. Soluble pigment is deep purple to black.

(9) Starch agar

Growth is vigorous, light reddish-orange, light reddish-purple to rufous. Powdery, olive-gray aerial mycelium is produced. Soluble pigment is faint yellow to faint yellowish pink. Zone of enzyme action on agar plate is 13–17 mm wide (15 days).

(10) Gelatine

Olivy light yellowish-brown growth is produced on the surface. Aerial mycelium is not produced. Brown and more diffusible light yellowish-green soluble pigments are produced. Liquefaction is weak to medium.

(11) Milk (37°C)

Growth is deep brown at 37°C, but creamy to light yellowish-brown and occasionally with pinkish tone at 25°C. Aerial mycelium is not produced. Soluble pigment is grayish yellow-brown at 37°C, but sometimes faint yellowish-brown with pinkish shade at 25°C. Coagulation begins in 3 days, and peptonization proceeds.

(12) No growth is observed on Paper Strip Synthetic Solution.

(13) Tyrosinase reaction is negative.

(14) Microscopic observation

On Synthetic agar and on Starch agar aerial hyphae produce open and colosed spirals. Conidia are short cylindrical, 0.5–0.9×0.9–1.2 μ.

(15) Dilution potency against *T. vaginalis* is less than 2 units, and haemolytic power is 2 units.

Comparing H-4650 with other known species, it is seen to have some characters in common with species belonging to the *S. ruber* group and *S. erythrochromogenes*, but differs from them in several ways. Though the color of the vegetative mycelium and the morphology of the aerial mycelium are the same, the color of the aerial mycelium of the *S. ruber* group is white or reddish-white and abundant, powdery, drab gray to olive gray aerial mycelium like that of strain H-4650 is never produced. This species is also clearly different from *S. erythrochromogenes* in the color of the vegetative mycelium and in soluble pigment production. Therefore H-4650 is considered a new species and named *S. griseoruber* n. sp., since the color of the aerial mycelium is gray as contrasted with the reddish color of vegetative growth. *S. griseoruber* n. sp. has the following characteristics: Aerial mycelium is powdery, drab gray to olive gray and produces open and closed spirals. Color of vegetative mycelium on synthetic media is reddish-orange to reddish-purple. Soluble pigment is brown, purple or black on organic media.

XV) Strains H-5192, H-4766

(1) Synthetic agar

Growth is spreading, colorless, thin, with abundant, powdery, white to pale smoke-gray aerial mycelium. Soluble pigment in not formed.

(2) Nutrient agar

Growth is wrinkled, at first creamy white, later yellowish-brown. Aerial mycelium is white, sometimes scanty in amount. Soluble pigment is absent or a trace.

(3) Starch agar

Growth is colorless to faint cream, with white to gray aerial mycelium. Soluble pigment is absent or a trace.

(4) Microscopic observation

Aerial hyphae produce abundant open spirals on various synthetic media.

(5) Dilution potency against *T. vaginalis* is less than 2 units in both strains, and haemolytic power is 0.

Further studies on various other media have shown that both strains resemble *S. albus* or *S. albidus*, species which belong to Waksman's *S. albus* group.

XVI) Strain H-4114

(1) Synthetic agar, Krainsky's agar

Growth is colorless, light cream to light olive-gray in reverse, penetrating deep into the medium. Abundant, powdery, light grayish olive aerial mycelium is quickly produced. Soluble pigment is not found.

(2) Calcium malate agar

Reverse is at first light yellow, later changing to light yellowish-brown to light pinkish brown. Powdery, grayish white aerial mycelium is abundantly produced. Faint pinkish-brown soluble pigment is produced slowly.

(3) Nutrient agar

Growth is golden-yellow in reverse and quickly covered with abundant, white aerial mycelium. Soluble pigment is doubtful.

(4) Blood agar (37°C)

Growth is vigorous, grayish-olive to dull yellow-green, with scanty, white to faint greenish-white aerial mycelium at top of slant. Soluble pigment is black. Haemolytic action is positive.

(5) Potato plugs

Growth is vigorous, elevated, wrinkled, and covered with powdery, gray aerial mycelium. Some darkening of plugs is seen.

(6) Starch agar

Reverse is faint yellow. Aerial mycelium is abundant, powdery, gray. Soluble pigment is absent or later, dark olive buff. Enzymatic zone on plate agar is 25 mm wide.

(7) Gelatine

Growth is colorless to white, later changing to dull yellow in reverse. Aerial mycelium is white, and soluble pigment is absent or faint yellow. Liquefaction is strong.

(8) Milk (37°C)

Growth is yellow at 37°C, but sometimes becomes faintly pinkish at 25°C. Aerial mycelium is scanty, white. Soluble pigment is dull yellow

to dull yellow-orange, but at 25°C sometimes faint buff to faint pink. Coagulation occurrs in 3 days and peptonization proceeds rapidly.

(9) Grows fairly well on Paper Strip Synthetic Solution.

(10) Microscopic observation

On various synthetic media aerial hyphae produce abundant open spirals, and show tuft-forming tendency in the margin.

(11) Dilution potency against *T. vaginalis* is less than 2 units, and haemolytic power is 0.

Comparing the above mycological characters with those of known species, H-4114 rather resembles *S. albogriseolus* Benedict et al[4]., though it has an ash-gray aerial mycelium and orange-colored growth on Milk. The carbon utilization of both strains is very similar.

XVII) Strains H-5119

(1) Synthetic agar

Growth is colorless, thin, rapidly producing abundant aerial mycelium. Aerial mycelium is at first white, later smoke gray to drab-gray. No soluble pigment is formed.

(2) Krainsky's agar

Growth is penetrating, pinkish with vandyke red to corinthian purple reverse. Aerial mycelium is at first white to pinkish-white, later whitish-gray. Soluble pigment is not formed.

(3) Nutrient agar

Growth is dull yellow in reverse and rapidly covered with whitish aerial mycelium. Soluble pigment is absent or trace.

(4) Potato plugs

Growth is elevated, wrinkled, reddish-orange to yellowish-orange. Aerial mycelium is powdery, olive-gray with pale reddish-orange portion. Some darkening of plugs is seen.

(5) Starch agar

Growth is at first colorless, but soon changes to pale purplish-pink. Aerial mycelium is rapidly produced, powdery, at first white, later deep olive-buff to drab-gray. Soluble pigment is not produced. Zone of enzyme action on agar plate is 4–7 mm wide (15 days).

(6) Gelatine

Growth is dull red-purple on the surface, reddish-cream in the medium. Aerial mycelium is slowly produced, white. Soluble pigment is missing or a trace. Liquefaction is strong.

(7) Milk (37°C)

Growth is at first pale pinkish, later faint yellow with pinkish tinge. Whitish aerial mycelium is produced. Soluble pigment is almost none or trace of yellowish-brown or pink. Coagulation is absent or very weak.

(8) Fair growth is seen on Paper Strip Synthetic Solution.

(9) Microscopic observation

On various synthetic media aerial hyphae form many open spirals, with

tuft-forming tendency in the margin.

(10) Dilution potency against *T. vaginalis* is less than 2 units, and haemolytic power is 0.

As seen in Table 3, carbon utilization is quite similar to that of strains H-5192 and H-4766.

Comparing H-5119 with other known species, it is found to have some characters in common with *S. californicus* and *S. albosporeus*, and seems to be most like *S. californicus* (Waksman and Curtis) Waksman and Henrici, though some differences in color of both the aerial and the vegetative mycelium are seen.

XVIII) Strains H-5055

(1) Synthetic agar

Growth is thick, colorless, light creamy to olive-buff. Aerial mycelium is white to gray. Soluble pigment is not formed.

(2) Nutrient agar

Growth is colorless, smeary, and no aerial mycelium is formed. Soluble pigment is trace or yellowish-brown.

(3) Starch agar

Reverse is pale yellow. Aerial mycelium is mouse gray to light drab, but later becomes hygroscopic and produces glossy, black portions. Soluble pigment is not produced.

(4) Microscopic observation

On Starch agar aerial hyphae produce a few open spirals and closed spirals. Conidia are cylindrical, 0.5–0.8×0.7–1.0 μ.

(5) Dilution potency against *T. vaginalis* is 2 units, and haemolytic power is 0.

The cultural characters on other media have also been studied. H-5055 seems to resemble somewhat *S. hygroscopicus* and the *S. diastatochrogenes* group. However, it is clearly different from the latter in the points of soluble pigment production, the hygroscopic nature of the aerial mycelium, and carbon utilization. From the hygroscopic nature of its aerial mycelium H-5055 seems to be most similar to *S. hygroscopicus* (Jensen) Waksman and Henrici, though we can find minor differences between them.

XIX) Strains H-4501, H-4496

(1) Synthetic agar

When newly isolated, growth is reddish-orange, but after repeated transfer it becomes first colorless to faint cream, later whitish-cream to olive-buff. H-4501 produces white aerial mycelium, and H-4496 does not. Soluble pigment is not found.

(2) Krainsky's agar

Growth is at first colorless to white, later reddish-orange in reverse. Yellowish or pinkish-white aerial mycelium is formed. Soluble pigment is

faint yellow to faint orange.

(3) Nutrient agar

H-4501 has at first grayish cream, later brownish growth, none or scanty, white aerial mycelium, and brown soluble pigment. On the other hand, H-4496 has colorless to faint yellow growth, and does not produce aerial mycelium or soluble pigment.

(4) Starch agar

Growth is colorless, apricot-orange, later vinaceous tawny in reverse. Aerial mycelium is cottony, white. Soluble pigment is absent or faint orange in old cultures.

(5) Microscopic observation

On various synthetic media aerial hyphae branch irregularly, and no spirals or whorls are observed.

(6) Dilution potency against *T. vaginalis* is 4 units in H-4501, and less than 2 units in H 4496; haemolytic power is 0 in both strains.

These and other cultural characters on various media show that both strains belong to Waksman's *S. ruber* group.

4. Carbon utilization of newly isolated anti-trichomonal Actinomycetes.

The above typical cultures were inoculated on to Pridham and Gottlieb's synthetic medium[37], each containing 21 different sugars as a sole source of carbon, and incubated for 10 days at 25°C. As they are presented in Table 3, the results show that strains belonging to same species are quite similar, or have at least the same tendencies, in carbon utilization. Glucose and Mannose are utilized by all strains, d-Fructose by all strains except H-4725, d Maltose by all except H-4592, Trehalose by all except H-3882, H-4684, and H-4490. Dulcit is not utilized by most of the strains. There is no common carbon utilizability among anti-trichomonal Actinomycetes.

5. Change in activity by repeated transfer.

As some of the cultures which were isolated in the autumn of 1953 and showed anti-trichomonal activity lost their activities later, the activity of 35 typical cultures was re-examined. Table 4 shows that strains belonging to the *S. diastatochromogenes* and *S. ruber* groups tend to loose their activity, while none of the strains belonging to the *S. griseus*, *S. reticuli*, *S. hachijoensis*, *S. lavendulae* and *S. naganishii* n. sp. groups lose their activity. Loss of anti-trichomonal activity and dilution potency are not always parallel, and, though strains belonging to the *S. ruber* group, such as H-4725 and H 4501, have a dilution potency of 4 to 8 units, they lose their activity, while strains belonging to the *S. griseus* and *S. lavendulae* groups do not lose their activity, though their dilution potency is only 2 units or less.

Table 3. Carbon utilization of Typical Anti-trichomonal Actinomycetes

C source / Strain	d (−) Xylose	l (+) Arabinose	l (+) Rhamnose	d (+) Galactose	Saccharose	Lactose	Raffinose	Inulin	Mannitol	Sorbitol	Inositol	Salicin	Na-acetate	Na-citrate	Na-succinate	Control
Streptomyces purpeofuscus n. sp.																
H–5080	++	++	(−)	++	++	+	+	(−)	(−)	(−)	(−)	(−)	(−)	(−)	+/−	(−)
S. fasciculus																
H–3882	++	+	++	++	(−)	++	(−)	(−)	++	−	(−)	(−)	(−)	+	+/−	(−)
S. griseus g.																
H–4038	++	++	(−)	++	+	+	−	−	++	−	−	+	+	+/−	+	−
S. reticuli g.																
H–5044	(−)	(−)	−	(+)	±	(+)	−	±	+/−	−	+/−	±	+	(+)	+	−
H–4684	(+)	(+)	−	+	(+)	−	−	−	+	−	+	−	(+)	(+)	++	−
H–4490	(−)	±	−	++	−	−	−	−	++	−	−	−	+	+	++	−
S. hachijoensis g.																
H–4278	(+)	−	±	++	(+)	±	(+)	+	±	±	++	−	±	(+)	+	(+)
H–4592	(+)	(+)	−	++	−	+	−	−	++	−	+	−	(+)	+	++	−
S. olivochromogenus																
H–3845	++	++	+	++	−	++	+	−	++	(−)	−	+	+/−	+	+	−
S. purpeochromogenus																
H–5059	++	+	−	++	++	++	−	−	−	−	−	+	+	±	+/−	−
H–4742	++	++	(−)	++	++	++	(−)	(−)	−	(−)	(−)	+	(−)	+	(−)	−
S. lavendulae g.																
H–4422	−	(−)	(−)	++	(−)	(−)	(−)	(−)	(−)	+	(−)	+	+	++	−	(−)
H–5115	−	(−)	(−)	−	(−)	−	(−)	(−)	−	(−)	−	(+)	+/−	(−)		(−)
H–5102	−	(−)	−	(+)	(−)	(−)	(−)	(−)	++	(−)	+	−	(+)	(+)	(−)	(−)
H–5002	+	++	(−)	+	(−)	−	−	(−)	(−)	(−)	−	−	(+)	+	−	(−)
H–4519	+	(−)	(−)	(−)	++	+	(−)	(−)	(−)	(−)	−	+	+	+	+	−
H–4517	+	(−)	(−)	(+)	++	(−)	(−)	(−)	(−)	(−)	(−)	+	+	+	+/−	(−)
H–4778	(+)	(+)	(−)	++	++	(−)	(−)	(−)	(−)	(−)	(−)	+	+	+	(+)	(−)
S. fradiae g.																
H–3904	++	+	+	++	(−)	++	(−)	(−)	++	−	(−)	(−)	+	(+)	+	(−)
H–3798	++	++	−	++	−	(+)	−	−	−	(−)	−	−	(−)	±	−	−
S. ruber g.																
H–4725	−	−	++	++	++	++	++	++	++	−	++	+	+	−	+	−
H–4501	++	−	++	++	++	++	++	++	++	−	++	(−)	+	+	+	−
H–4496	++	±	++	++	+	++	++	++	++	−	++	±	−	−	(−)	−
S. farinosus																
H–4156	(+)	−	++	+	++	±	±	(−)	++	(−)	−	(−)	+	+	( )	

Strain \ C source	d (−) Xylose	l (+) Arabinose	l (+) Rhamnose	d (+) Galactose	Saccharose	Lactose	Raffinose	Inulin	Mannitol	Sorbitol	Inositol	Salicin	Na-acetate	Na-citrate	Na-succinate	Control
S. naganishii n. sp. g.																
H-4871	++	++	++	++	(−)	++	++	−	++	−	++	+	++	++	+	−
H-4869	++	++	++	+	(−)	++	++	(−)	++	(−)	++	+	+	+	(+)	(−)
S. diastatochromogenes g.																
H-4185	++	(+)	−	++	+	++	++	−	++	++	++	+	(−)	++	+	−
H-4630	(+)	+	+	++	++	++	++	++	−	−	++	(−)	+	(−)	(+)	−
H-4678	+	(−)	++	++	++	++	++	++	++	−	++	+	++	+	+	−
H-4413	±	−	−	(+)	++	+	±	−	++	−	++	−	−	+	+	−
H-4640	++	++	+	+	+	++	++	+	++	(−)	++	++	+	+	++	(−)
S. griseoruber n. sp.																
H-4650	+	+	++	(+)	−	++	(−)	−	−	−	+	+	+	(−)	(−)	−
S. albus g.																
H-5192	+	++	++	++	++	++	(−)	−	++	−	++	++	+	(−)	++	−
S. albogriseolus																
H-4114	++	+	++	++	++	++	(−)	(−)	++	±	++	+	+	+	++	(−)
S. californicus																
H-5119	++	+	++	++	(+)	++	++	(−)	++	−	++	+	+	+	(+)	(−)
S. hygroscopicus																
H-5055	−	(+)	−	++	++	(+)	−	−	−	−	−	−	(−)	−	−	−

* ++, +: Good growth and positive utilization.
(+), ±: Doubtful utilization.
(−), −: No growth or faint growth, and negative utilization.

6. *Activities against trichomonads other than T. vaginalis.*

We examined the activities of anti-trichomonal Actinomycetes which had been selected by using *T. vaginalis* as test organism against other trichomonads, such as *T. foetus*, *T. elongata* and *T. tenax*. But owing to difficulties in getting pure and vigorous growth of the last two species in vitro, we could not apply the "Diphase method" to these microorganisms. Therefore only the activity against *T. foetus* Asa No. 48 is described here. As is shown in Table 5, the activity against *T. foetus* is identical with that against *T. vaginalis*, except for strains H-3902, H-3798, and ATCC 3381. 9 strains, reported in the previous section as having lost their activity, were found to have no activity against *T. foetus*, either.

7. *Antibiotic spectrum of anti-trichomonal Actinomycetes.*

Zaher et al.[53] reported that, among their 20 anti protozoal Action-

Table 4. Change in Anti-trichomonal Activity by Repeated Tranfer.

Species or Group	Strain	1953 Autumn	1955 Winter	Species or Group	Strain	1953 Autumn	1955 Winter
Streptomyces purpeofuscus n. sp.	H–5080	0/a*	0/a	*S. fradiae* g.	H–3798	0/a	0/a
S. fasciculus	H–3882	0/a	0/a	*S. ruber* g.	H–4725	0/a	30/1
S. griseus g.	H–4038	0/a	0/a	*S. farinosus*	H–4156	0/a	8/1
〃	H–3902	0/a	0/a	*S. naganishii* n. sp. g.	H–4871	0/a	0/a
S. reticuli g.	H–5044	0/a	0/a	〃	H–4869	0/a	0/a
〃	H–4490	0/a	0/a	*S. diastato-chromogenes* g.	H–4185	0/a	0/a
S. hachijoensis g.	H–4278	0/a	0/a	〃	H–4630	0/a	7/1
〃	H–4592	0/a	0/a	〃	H–4678	0/a	6/1
S. olivochromo-genus	H–3845	0/a	10/1	〃	H–4413	0/a	4/1
S. purpeochro-mogenus	H–5059	0/a	0/a	〃	H–4640	0/a	1/1
S. lavendulae g.	H–4422	0/a	0/a	*S. griseoruber* n. sp.	H–4650	0/a	5/1
〃	H–5115	0/a	0/a	*S. albus* g.	H–5192	0/a	0/a
〃	H–5102	0/a	0/a	*S. albogriseolus*	H–4114	0/a	10/1
〃	H–5002	0/a	0/a	*S. californicus*	H–5119	0/a	9/1
〃	H–4519	0/a	0/a	*S. hygroscopi-cus*	H–5055	0/a	0/a
〃	H–4517	0/a	0/a	*S. ruber* g.	H–4501	0/a	4/1
〃	H–4778	0/a	0/a	〃	H–4496	0/a	1/1
S. fradiae g.	H–3904	0/a	0/a	Control		5/1–30/1	10/1–20/1

* For explanation of ratios See Table 2.

mycetes, 12 cultures showed anti-bacterial activity against 1 to 4 species of bacteria. Anti-microbial activities of our newly isolated anti-trichomonal Actinomycetes have also been examined. As test organisms we selected *Mycobacterium phlei* and *Mycobact.* 607 as acid-fast organisms, *Micrococcus aureus* 209 P, *B. subtilis*, and *B. agri*, as Gram positive bacteria, *Escherichia coli*, Streptomycin-fast-*E. coli* and Streptothricin fast-*E. coli* as Gram negative bacteria, and *Saccharomyces sake* and *Candida albicans* as fungi.

As shown in Table 6, 115 out of 138 strains are active, and only 23 strains do not show any anti-microbial activity. In general, many strains showed anti fungal or anti-Gram positive bacterial activity, while those active against Gram-negative bacteria were comparatively few and limited to the *S. reticuli*, *S. hachijoensis*, and *S. lavendulae* groups, and *S. californicus*. Inactive strains are scattered in many species. Although strains belonging to the *S. naganishii* n. sp. group, the *S. ruber* group, and *S. purpeochromogenus* showed the same antibiotic spectrum, strains belonging to

Table 5. Comparative activity of strains of Actinomycetes against *Trichomonas voginalis* and *T. foetus Asa No.* 48.

Species or Group	Strain	*T. vaginalis*	*T. foetus*	Species or Group	Strain	*T. vaginalis*	*T. foetus*
S. purpeofuscus n. sp.	H-5080	0/a	0/a	*S. lavendnlae* g.	0-20-316	0/a	0/a
S. fasciculus	H-3882	0/a	0/a	*S. fradiae*	H-3904	0/a	0/a
S. griseus g.	H-4038	0/a	0/a	〃	H-3798	0/a	7/1
〃	H-3902	0/a	2/1	〃	NRRL B-1195	0/a	0/a
〃	2463	0/a	0/a	〃	3535	0/a	0/a
S. reticuli g.	H-5044	0/a	0/a	*S. ruber* g.	H-2491	0/a	0/a
〃	H-4490	0/a	0/a	*S. naganishii* n. sp. g.	H-4871	0/a	0/a
〃	S. 299-5	0/a	0/a	〃	H-4869	0/a	0/a
〃	Z-1-6	0/a	0/a	*S. diastato-chromogenes* g.	H-4158	0/a	0/a
〃	H-365	0/a	0/a	*S. albus* g.	H-5192	0/a	0-a
S. hachijoensis g.	H-4592	0/a	0/a	〃	ATCC 3381	0/a	4/1
〃	H-2609	0/a	0/a	*S. hygroscopi-cus*	H-5055	0/a	0/a
S. purpeochro-mogenus	H-5059	0/a	0/a	〃	6A-704	0/a	0/a
S. lavendulae g.	H-4422	0/a	0/a	*S. aureofaciens*	NRRL 2209	0/a	0/a
〃	H-5115	0/a	0/a	*S. flavochro-mogenes*	H-3206	0/a	0/a
〃	H-5102	0/a	0/a	*S. thioluteus*	26A	0/a	0/a
〃	H-5002	0/a	0/a	G-167	G 167	0/a	0/a
〃	H-4519	0/a	0/a				
〃	H-4517	0/a	0/a				
〃	H-4778	0/a	0/a				
〃	ATCC 8664	0/a	0/a	Control		10/1-20/1	10/1-12/1

the *S. reticuli*, *S. lavendulae*, *S. diastatochromogenes*, and *S. griseus* groups showed various antibiotic spectrums, so that a great number of anti-trichomonal antibiotics must exist.

8. *Classification of Anti-trichomonal Actinomycetes.*

Many authors, such as Krassilnikov, Waksman and Henrici, have tried to make a classification of the Actinomycetes using various characters as principal keys.—Some employed morphological characters, that is branching and shape of conidiophores or conidia, cultural characters, including saprophytism and parasitism, soluble pigment production, color of growth, and color or formation of aerial mycelium, as principal keys; while others tried to attach more importance to biochemical characters such as carbon or nitrogen utilization, serological reactions, antibiotic relations, phage speci-

Table 6. Number of strains of Actinomycetes active against various groups of microorganisms.

Species or Group \ Active Against	APNF	APN	APF	ANF	PNF	AP	AN	AF	PNF	PF	NF	A	PF	N	None	Total
S. purpeofuscus n. sp.						1										1
S. fasciculus															1	1
S. griseus g.								1		1			1	3	2	8
S. reticuli g.	11			2	7			1								21
S. hachijoensis g.				1						1						2
S. purpeochromogenus													2			2
S. lavendulae g.	17		5	2	9			4	3	4	1	3	1	14	4	67
S. fradiae g.															1	1
S. ruber g.															4	4
S. farinosus															1	1
S. naganishii n. sp. g.			6													6
S. diastatochromogenes g.										3			6	1	9	19
S. griseoruber n. sp.						1										1
S. albus g.															1	1
S. albogriseolus												1				1
S. californicus									1							1
S. hygroscopicus										1						1

A: Acid-fast bacteria
N: Gram negative bacteria
P: Gram positive bacteria
F: Fungi

ficity, enzymatic activities, and so forth. Baldacci et al[1)2)]., Hesseltine et al[14)]., Burkholder and sun[6)] and Gause[9)] discussed the matter in general and recently Couch[7)] has proposed two new additional genera, *Actinoplanes* and *Streptosporangium*.

After careful microscopic observation of 33 typical anti-trichomonal Actinomycetes on various synthetic media, we concluded, as Dr. Hesseltine

has stated in his paper, that the morphology of a particular strain of Actinomycetes is essentially the same on any medium where typical sporulation occurs. So we divided the above 172 strains into 4 sections; those having straight aerial hyphae without spirals or whorls, whorl formers, spiral formers, and those showing no characteristic figures. As for spiral formers we decided to divide them into two sub-sections for the sake of convenience: predominantly closed spiral producers and predominantly open spiral producers. Frequently, however, we could not sharply demarkate these types, and sometimes they were changed by culture conditions. For the separation of species or groups within a section, we selected the color of the aerial mycelium on synthetic media, the production of brown soluble pigment on organic media, and the color of growth on synthetic media as primary, secondary, and tertiary keys respectively. The number of strains in each group is shown in ( ).

I. Aerial hyphae straight, tuft forming tendency in the margin. No whorls or spirals.

(1) Aerial mycelium gray.

A. Light purple, reddish purple, purplish-brown, sometimes yellowish-brown soluble pigment on protein media.
..... *S. purpeofuscus* n. sp. (1)

B. Does not produce brown soluble pigment.
..... *S. fasciculus* (1)

(2) Aerial mycelium pale yellowish green.

A. Brown soluble pigment on protein media.
..... ATCC 3309* (1)

B. Does not produce brown soluble pigment.
..... *S. griseus* group (10)

II. Aerial hyphae straight, whorls produced.

(1) Cottony aerial mycelium white, light tan or pale pink.

A. Brown soluble pigment on protein media.
..... *S. reticuli* group (28)

B. Does not produce brown soluble pigment.
..... *S. hachijoensis* group (5)

III. Spiral-shaped conidiophores, produced on aerial hyphae.

1. Predominantly closed spirals produced.

(1) Aerial mycelium gray.

A. Brown soluble pigment on protein media.

* Aerial hyphae of ATCC 3309 (*S. aureus*) are straight, and no spirals have been observed on Czapek's agar, Starch agar, and Krainsky's agar, though *S. aureus*[46] is reported to have numerous long spirals, according to the original description.

i) Growth colorless, yellowisn brown, brown or deep brown.
..... *S. olivochromogenus* (1)
ii) Growth light purple to purplish-black.
..... *S. purpeochromogenus* (1)
B. Does not produce brown soluble pigment.
..... *S. aureofaciens* group (1)
(2) Aerial mycelium pale pink.
A. Brown pigment on protein media.
..... *S. lavendulae* group (72)
B. Does not produce brown soluble pigment.
..... *S. fradiae* group (7)
2. Predominantly open spirals or compact spirals produced.
(1) Aerial mycelium white.
A. Brown soluble pigment on protein media.
i) Growth reddish.
..... *S. ruber* group (3)
B. Does not produce brown soluble pigment.
..... *S. farinosus* (1)
(2) Aerial mycelium gray.
A. Brown soluble pigment on protein media.
i) Abundant compact spirals produced on aerial hyphae.
..... *S. naganishii* n. sp. group (6)
ii) Growth colorless, white, light yellow or yellowish-brown.
..... *S. diastatochromogenes* group (19)
iii) Growth colorless, reddish-orange or reddish purple.
..... *S. griseoruber* n. sp. (1)
B. Does not produce brown soluble pigment.
i) Growth colorless to creamy
..... *S. albus* group (3)
ii) Growth colorless, white, light yellow or yellowish-brown.
..... G 167** (resembling to *S. cacaoi*) (1)
iii) Growth colorless, light yellow or light pinkish brown.
..... *S. albogriseolus* (1)
iv) Growth purple to pink to red.
..... *S. californicus* (1)
(3) Aerial mycelium gray, but on certain media moist with dark, glistening patches.
..... *S. hygroscopicus* (2)

IV. No characteristic features of aerial hyphae.
(1) Very limited aerial mycelium production on various media.
A. Deep brown soluble pigment on protein media.

** This strain was supplied by Dr. Sakai of Fuculty of Agricultural Chemistry of the same university as typical anti-Mycobact. 607 Actinomycete.

i) Growth yellow to yellowish-brown.
..... *S. flavochromogenes* (1)

B. Faint yellowish brown soluble pigment.
..... *S. thioluteus* (2)

(2) Aerial mycelium white.

i) Growth colorless, light yellow or reddish-orange.
..... *S. ruber* group (2)

SUMMARY

1. The mycological characters of 172 anti-trichomonal Actinomycetes, which had been selected from 1244 typical Actinomycetes, were studied, and their belonging 9 groups, 12 specics, and 3 new species is described. Among them the *S. lavendulae*, *S. reticuli*, *S. diastatochromogenes*, and *S. griseus* groups had large numbers of strains as shown in Table 1. However, some of the cultures of the *S. diastatochromogenes* group and the *S. ruber* group quickly lost their activity after repeated transfers on synthetic medium (Table 4).

2. Morphologically these Actinomycetes may be divided into four sections, and, as shown in the text, further classified by using the color of the aerial mycelium, soluble pigment production, and the color of vegetative growth as keys.

3. Strains H-5080, H-4871, and H-4650 were considered to be new species and were named *S. purpeofuscus* n. sp., *S. naganishii* n. sp., and *S. griseoruber* n. sp., respectively.

4. The antibiotic spectrum, the dilution potency, and the haemolytic power of these Actinomycetes were examined.

5. Carbon utilization of typical anti-trichomonal Actinomycetes was studied (Table 3).

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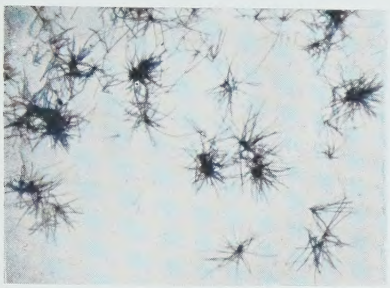

Fig. 1. *Streptomyces purpeofuscus* n. sp. grown on Synthetic agar, × 200

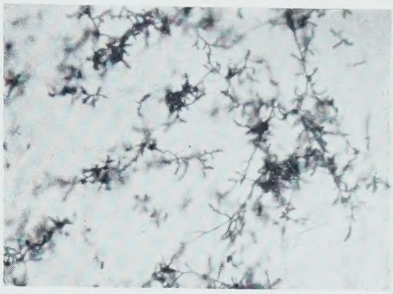

Fig. 4. H–4185, grown on Starch ager, a strain of the *S. diastatochromogenes* group, × 200

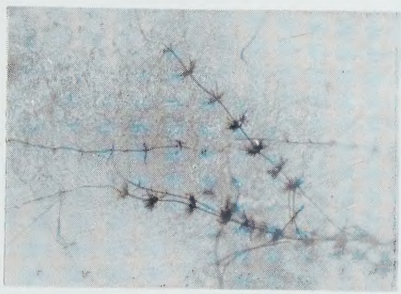

Fig. 2. H–4278, of the *S. hachijoensis* group, × 200

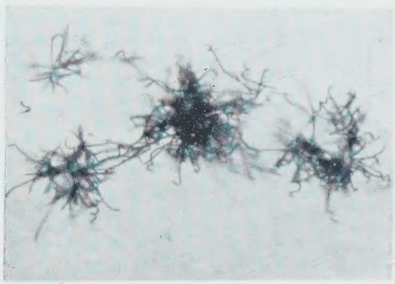

Fig. 5. *S. griseoruber* n. sp., grown on Synthetic agar, × 200

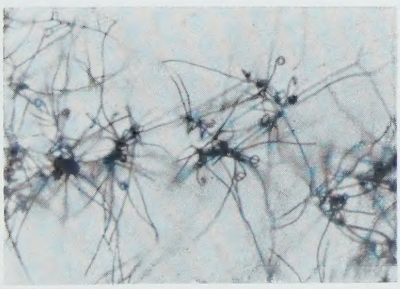

Fig. 3. H–4422, of the *S. lavendulae* group, × 200

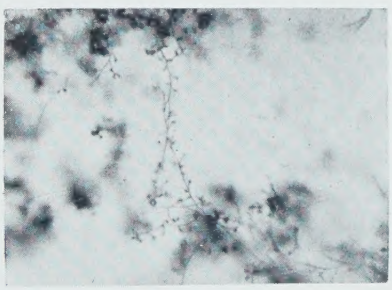

Fig. 6. *S. naganichii* n. sp., grown on Synthetic agar, × 200

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CONTENTS

Y. MORIMURA, T. NIHEI and T. SASA: Outdoor Bubbling Culture of Some Unicellular Algae..... 173

T. SASA, Y. MORIMURA and H. TAMIYA: Seasonal Variation of Growth Rate of Various Strains of Unicellular Algae under Natural Light- and Temperature-Conditions. 183

A. WATANABE, R. ITO and T. SASA: Effect of Various Chemicals upon the Growth of *Daphnids*. 190

H. IIZUKA and T. YAMAGUCHI: Studies on *Aspergillus usamii mut. shiro-usamii* and its Industrial Application. 194

T. YAMAGUCHI: Studies on the Anti trichomonal Actinomycetes and their Classification..... 201